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The Prevalence of Gastrointestinal Parasitic Infections and Assessment of Therapeutic Procedures among Ruminants

MASTER'S THESIS

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Declaration

I hereby declare that I have done this thesis entitled “The Prevalence of Gastrointestinal Parasitic Infections and Assessment of Therapeutic Procedures among Ruminants” independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

April 2021, Prague

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Dominika Roudná

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Abstract

The research of the diploma thesis was focused on monitoring the occurrence of developmental stages of parasites in sheep and goats on selected farms on the island of Aruba. It was a pilot project focused on the demonstration of protozoa and helminths and further verification of the effectiveness of the chemical preparation and plant extract, with the declaration of effectiveness against coccidiosis. In collaboration with Riem van den Berg DVM, microbiologist, interim Head of the Veterinary Service in Aruba, research was carried out in field conditions and in the laboratory and the presence of parasitic species of coccidia *Eimeria* spp. nematodes and *Trichuris* spp. The research took place in the period February 2019-December 2020 on selected sheep and goat farms, in cooperation with the Veterinary Diest Aruba and local breeders. There were four farms, where a total of 202 samples of sheep and goat droppings were taken and subsequently processed in the laboratory. Initially, the research was focused on parasitological monitoring, to determine parasitic agents using the flotation-centrifugation method. In the next stage of the research, sampling was targeted at farms where the positive occurrence of parasites in individual animals was confirmed, followed by laboratory examinations before and after the application of antiparasitics. The aim was to demonstrate the therapeutic efficacy of the veterinary medicinal product Vecoxan® (Inc. Elanco, UK), containing diclazuril and plant extract Emanox® (Biokron Ltd. comp., Czech Republic) in selected localities of the Caribbean island based on laboratory results. Manure collection was performed individually, from the youngest individuals raised on farms, before and after administration of medicinal products Vecoxan®, containing diclazuril, was administered to the first two farms B (Sero Alejandro) and C (Sero Grandy). On other farms D (Jaburubari) and E (San Nicolas) the natural plant extract Emanox® was applied in control and monitored groups of animals. The efficacy of both products was statistically evaluated, similar to the weight gain of animals after drug administration. The results initially showed a 100% occurrence of coccidia of the genus *Eimeria* spp. in sheep and goats, on all farms.

After administration of the medicinal products, a reduced intensity of infection of developmental stages of Eimeria oocysts was statistically demonstrated in both monitored anticoccidials and animal species, ie in the medicinal product Emanox® ($p = 0.001896$ in

sheep, p = 0.014095 in goats). Nevertheless, in the treatment of coccidiosis, a higher efficacy of the anticoccidial agent containing diclazuril was found, when there was a significant decrease in the intensity of infection in sheep (p = 0.000135), compared to goats (p = 0.002633). For product D, a statistically significant difference (p = 0.15239) was found in the reduction in infection intensity between goats and sheep. In addition, the mean pre-drug weight was in sheep (Emanox® - 17.75 ± 6.90 kg, Vecoxan® -77 ± 2.46 kg) and goats (Emanox® - 14.50 ± 4.53, Vecoxan® - 13.27 ± 2 , 90 kg) and administration in sheep (Emanox® - 19.94 ± 6.86 kg, Vecoxan® - 14.82 ± 1.87 kg) and goats (Emanox® - 16, 94 ± 4.43 kg, Vecoxan® - 15, 68 ± 2, 68kg). Thus, no statistically significant differences between sheep and goats either in the initial average weight or in the average weight after administration of the product. Differences in animal weights after drug administration were in sheep (Emanox® - 2.19 ± 0.26 kg, Vecoxan® - 3.05 ± 2.04 kg) and goats (Emanox® - 2.44 ± 0.63 kg, Vecoxan® - 2 , 41 ± 1.59 kg).

Given the growing number of animals reared on the island without any medicinal products, attention should be focused on biosecurity in the youngest age group of sheep and goats, as coccidiosis has a high prevalence and could affect the economics of small-scale farming animal farms in Aruba.

Key words: parasites, sheep, goats, antiparasitic treatment, biosecurity

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List of the abbreviations used in the thesis

BBSAI	Barbados Blackbelly Sheep Association International
WHO	World Health Organization
GIN	Gastrointestinal Nematodes
FAO	Food and Agriculture Organization
FEC	Faecal Egg Counts
OPG	Oocysts per Gram
EPG	Egg per Gram
FECPAK^{G2}	Faecal egg count in package
FLOTAC	New multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans
PABA	Para-aminobenzoic acid

1. Introduction and Literature Review

1.1. Introduction

Parasitosis in ruminant farms has always significantly affected the economic parameters of animal husbandry, so even today, great emphasis should be placed on ensuring welfare and biosecurity conditions in animals. Sheep and goat management should allow animals access to pasture for most of the season and allow them to move naturally. Pasture was often a major source of parasitic infections in ruminants, and the survival of oocysts of coccidia and gastrointestinal nematode eggs was dependent on different environmental conditions. Knowledge of the biological cycles of parasites has also become an important moment in preventing the spread of developmental stages of parasites and has enabled the setting of external and internal biosecurity in grazing conditions. Infections with gastrointestinal stages of parasites in the temperate zone used to be common and were resolved by surface dehelmintisation, which unfortunately led to resistance to the administered veterinary medicinal products.

The spread of parasitic agents in tropical and subtropical areas, such as the Caribbean, was of great importance. Thanks to favorable abiotic factors, such as temperature and humidity, the prevalence of parasitosis could reach up to 100% of the values, and clinically, coccidiosis and helminthosis manifested themselves in frequent deaths of animals, especially young age groups. Coccidia of the genus Eimeria were included in one of the most species-specific groups of parasitic protozoa, subspecies Apikomplexa, class Sporozoa, subclass Coccidia. The Sporozoa class included many intracellular forms of parasites and actively penetrated host cells. Coccidiosis of small ruminants, caused by parasitic protozoa of the genus Eimeria, posed a risk in sheep and goat farms, especially for young at the age of 4-6 months. Under adequate conditions, oocysts sporulated at two to five days at 24° C to 32° C. Therefore, more susceptible sheep or goats could be infected with massive numbers of infectious sporocysts under these optimal conditions. For a long time, based on morphology, coccidia species of the genus Eimeria were considered identical in goats and sheep.

The spread of parasitic infections in these climatic conditions has always had a major economic impact on meat production. This also led to collaboration with colleagues from Aruba, and this project focused on determining the prevalence of parasitosis using laboratory methods, to set preventive measures and also to verify the effectiveness of appropriate anticoccidials in different sheep and goat farms, on small private farms, on the island of Aruba.

1.2. Literature Review

1.2.1. Aruba Overview

The island of Aruba was located in the Caribbean Sea, 29 kilometers north of the Venezuelan peninsula of Paraguay. Together with the islands of Bonaire and Curasao, it was included in the so-called ABC Islands, which were about 80 kilometers away. Aruba became an independent self-governing part of the Kingdom of the Netherlands in 1986. The capital and at the same time the only international port on the island - the city of Oranjestad.

The area of the island was 180 km^2, with a population of about 112,300. The island was characterized by mostly low altitude and was formed mainly from igneous rocks lined with limestone deposits and lined with coral reefs. The highest point of the island was described as Mount Jamanota, which rose to a height of 189 meters above sea level. The soil in Aruba was barren, with little or no natural irrigation. Desalination of seawater yielded the majority of drinking water on the island (Britannica-Aruba 2018).

The population was ethnically mixed, often combined with Spanish, Dutch and African ancestors. The official languages used were Dutch and the so-called papiament, a Creole language that evolved from Portuguese, Spanish and Dutch (Aruba.com).

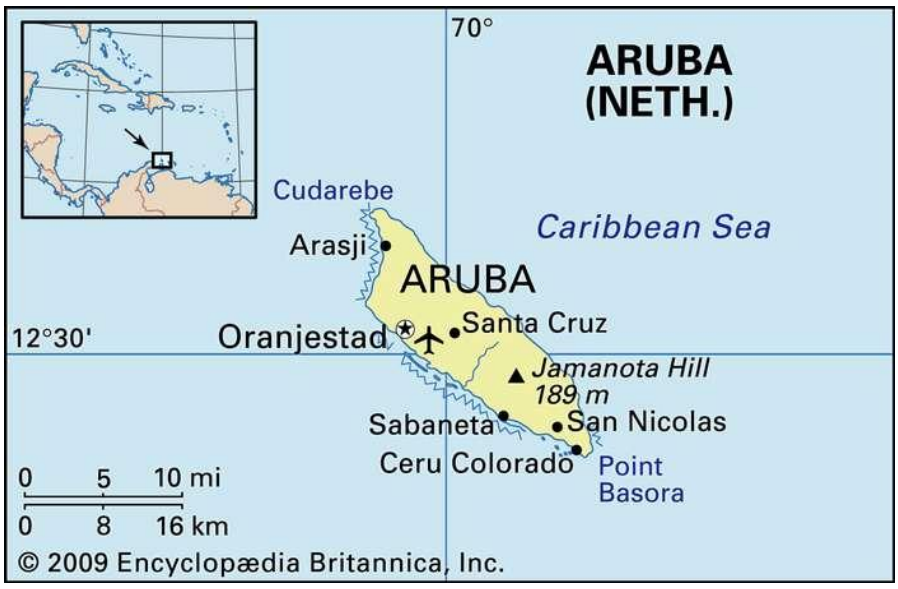

Figure1: Division and location of Aruba (Orig. Encyklopedie Britannica, Inc)

1.2.1.1. Aruba – climate

The island of Aruba was located in a tropical area, where the climate was divided into two alternating seasons, ie rainy and dry. The rainy season lasted from about September to January and was followed by a dry season that began in February and ended in June. Precipitation reached approximately 500 millimeters per year, with an average annual relative humidity of approximately 76%.

The temperature ranged from 29-31° C throughout the year, ie (84-88° F). Aruba, compared to other Caribbean countries, had a predominantly arid climate, as evidenced by vegetation consisting primarily of shrubs and cacti.

The island was located on the southern edge of the hurricane zone, although in these latitudes their occurrence was rare and their activity could manifest itself during August to October. The last tropical storm to hit the island was called Felix (September 2007) and a year later called Omar (October 2008), as described by Climatestotravel, Meteo.aw.

1.2.1.2. Hydrographical situation

The island´s groundwater resources were limited to predominantly brackish waters. The supply and poor quality of groundwater, as well as the lack of new sources of surface water, caused the production of drinking water to be started using seawater treatment. In many countries, groundwater has been of great importance to society, so its quality has always been carefully controlled. The bractic composition of groundwater depended on seawater intrusion, irrigation and a semi-arid climate. The largest amount of drinking water was obtained by desalination of seawater. In agricultural localities, with a lack of water, the problem of Agriculture, Livestock and Fisheries (Sambeek 2000) has helped with this problem.

1.2.1.3. Agriculture and Production

Aruba was characterized by an open economic system, where the largest percentage of the country´s income was provided mainly by tourism. Poor soil quality on the island and low rainfall limited agricultural development, yet *Aloe vera* cultivation and livestock farming, mostly sheep and goats, contributed to economic returns (globaltenders.com).

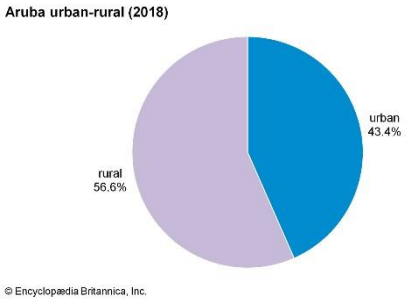

Figure 2: Aruba urban-rural (Orig. Encyklopedie Britannica, Inc)

Aloe vera plants were first planted in the desert in Aruba in 1850. The Netherlands, Brazil and Venezuela became the main trading partners for animal trade. The USA became a major importer of production to the island of Aruba, while exports went mainly to Colombia (Worldbank.org).

Table 2: Aruba Inport/export

Reporter Name	Partner Name	Year	Trade Flow	Product Group	Export (US$ Thousand)	Import (US$ Thousand)
Aruba	Brazil	2017	EXPIMP	Animal	0	13598,11
Aruba	Colombia	2017	EXPIMP	Animal	0	68,58
Aruba	Netherlands	2017	EXPIMP	Animal	0	16877,17
Aruba	Suriname	2017	EXPIMP	Animal	0	89,54
Aruba	Unspecified	2017	EXPIMP	Animal	1019,6	20373,89
Aruba	United States	2017	EXPIMP	Animal	0	52404,54
Aruba	Venezuela	2017	EXPIMP	Animal	7,01	3670,84
Aruba	World	2017	EXPIMP	Animal	1026,61	107082,67
Aruba	Europe & Central Asia	2017	EXPIMP	Animal	0	16877,17
Aruba	Latin America & Caribbea	2017	EXPIMP	Animal	7,01	17427,07
Aruba	North America	2017	EXPIMP	Animal	0	52404,54

1.2.2. Sheep and goats in Aruba

Sheep were brought to the island during the early colonial period, when both domesticated and wild populations survived on the island and were represented in much smaller numbers and less adaptable in the wild than local breeds of goats. The main differences can be observed in the localities that the animals preferred. Lowlands suited sheep, in contrast to goats, which greatly preferred more mountainous terrain (Buurt 2012).

The Barbados blackbelly sheep and Dorper sheep black head breeds were most often bred in the tropical part of America. At present, the animals were imported mainly from Latin America and subsequently crossed here.

The Barbados blackbelly breed was characterized by high fertility, in contrast to the Dorper sheep breed, which was resistant to heat, and so on the island of Aruba was created by crossing the ideal combination for breeding these species.

Goats were brought to the island in early colonial times and became the main livestock species (Freitas et al. 2005). The breeding of these animals has been very efficient, with slow metabolism, low water consumption and low food resources (Campbell and Donlan 2005). Most farmers on the island bred the Boer breed.

Thanks to these characteristics, goats prospered in conditions that were very difficult for the survival of other herbivore species, especially in semi-arid areas, where goats managed to survive even under difficult conditions of the island state (Veerbeek 2016). Goats could graze almost any vegetation and were included among the so-called non-selective grazers. Since 1/3 of the island was surrounded by the Arikok National Park, where three species of plants could be seen, namely *Acacia tortuosa* (Arikok National Park 2015-II), *Stenocereus griseus* (Mexican organ pipe) and *Opuntia wentiana*, they became goats an ideal animal for breeding on this island.

1.2.2.1. Dorper Sheep Blackhead

The Dorper sheep blackhead breed developed in the 1930s mainly from the Dorset Horn and Blackheaded Persian breeds. The local South African breed Ronderib Afrikaner had a partial share in the breeding of the breed.

Dorset Horn was one of the so-called shortwave meat breed, while Blackheaded Persian were furry and belonged to the group of so-called thick-backed breeds. The aim of breeding was to ensure better carcass quality. Over several generations, a homogeneous population combining the desired characteristics of the starting breeds was bred. The beginnings of breeding began in South Africa, the breed further spread in North America, Brazil and Australia. In Europe, then in Germany, mainly in Baden-Württemberg.

Dorper sheep blackhead was one of the most prolific breeds of so-called hornless sheep. It was characterized by a long period of reproduction, which was not seasonally limited. The breed was resistant to extreme high and low temperatures and extremely undemanding, adapted to free grazing, capable of long marches for grazing. The animals were included in the breeding at the age of 12-14 months. The rams were able to reproduce all year round. The breed was further characterized by an exceptional growth rate reaching 36 kg [ie 80 pounds] in three and a half to four months and also had good maternal abilities and easy and trouble-free births.

Their lambs were energetic, with a high ability to survive, with the young growing rapidly and being well muscled. Females were also able to give birth to three lambs in two years. Dorper was persistent, tolerant of higher temperatures, insects and could thrive in conditions where other breeds would barely survive. A strong and so-called non-selective grazer, it could graze a wide range of forage types on pasture or was used for weed control in this environment (dorpersheep.org, afs.okstate.edu; Sambraus 2006).

Figure 3: Dorper sheep blackhead (Select Genes Ltd)

1.2.2.2. Barbados Blackbelly

The Barbados blackbelly breed originated and developed on the island of Barbados, in the Caribbean. It was created by crossing breeds (crosses of African hair sheep and European wooled breeds), which was brought in the mid-16th century, when Barabados was colonized by the English. Regardless of the origin of their European

wooled breeds of ancestors, the Barbadian sheep has moved away from the uncertain beginnings of breeding and has taken an important place in Barbadian agriculture.

The sheep were fully fertile in crossbreeding with other breeds, the breed was extremely resistant in humid and warm areas, where their breeding was very demanding. Adult ewes were characterized by high fertility, reproduction took place throughout the year and the females had twins or triplets and, under suitable conditions, twice a year. The average lambing rate to range between 1.50 to 2.30 lambs per ewe lambing. Blackbells are excellent foragers, had high resistance to disease and higher tolerance to internal parasites, without the need for chemical treatment. In many parts of the USA, it was not necessary to perform deworming, if the so-called pasture rotation was used, in animals with quality genetic parameters. The breed matured slowly and reached its ideal weight in about two years. At that time, the ewes weighed 38-45 kg (85-100 pounds) and the rams weighed about 45-59 kg (100-130 pounds). Sheep with a smaller body constitution tolerated better warm climates and a smaller carcass was also desirable for farmers, especially if they did not have the opportunity to treat the hull after slaughter by cooling. The taste of the meat was excellent, compared to the breeds on the Czech market, which was caused by less fatness of the meat.

Barbados Blackbelly Sheep Association International (BBSAI) controlled the census, numbers of animals, in America. African descent (unusual in America) and adaptation to tropical climates (unusual for sheep) provided the breed with significant genetic value and productive significance (afs.okstate.edu; blackbellysheep.org; livestockconservancy.org).

Figure 4: Barbados blackbelly sheep (Carol Elkins)

1.2.2.3. Boer goat

The breed probably originated in South Africa and was derived from Hottentot goats crossed with Nubian goats, including goats imported from Europe and India. The initial population was a mottled, small body frame. At the beginning of the 1940s, a small group of farmers set themselves the common goal of breeding a breed of goats with excellent meat parameters. In 1959, they formed an association, which in 1964 introduced the Performance Control. The first targeted import of peanuts from Namibia to Germany took place in 1977, in order to raise them to feed lions in zoos. Thus came the idea of breeding a meat breed of goats. The Boer have become the most productive meat breed of goats, with millions of Boer goats bred throughout South Africa, as well as in Australia and New Zealand, the United States and Canada, the United Kingdom, Germany and elsewhere.

Boer were appreciated for their size, fast weight gain, carcass quality, durability and flexibility, as well as high fertility, good maternal qualities, calm temperament. They often produced twins or triplets and could muddy twice in three years. The kids had very high daily gains, in the castrates they reached a higher live weight of more than 100 kg and without a carcass yield of about 50%, even without the addition of the core. The meat was tasty and tender, with a typical goat flavor. Leather was used to make shoes, gloves and to bind books. Compared to other breeds, goats had a less pronounced odor. The breeding importance of this breed in our conditions has often not been fully appreciated (britannica.com; Sambraus 2006).

Figure 5: Boer goat (James Marshall)

1.2.3. Important species of parasites

Parasites have always reached smaller to microscopic dimensions and for the whole or part of their life or developmental cycle they lived on the body or inside another body (host) and nourished at its expense (Zachovalová 2005). Parasitic organisms received food from the host. Those individuals who lived inside the host organism were referred to as endoparasites, if they lived on the surface of the body of the host organism, they were so-called ectoparasites.

Endoparasites included individuals who lived, for example, in their gastrointestinal tract or respiratory tract (see Figure 6), while ectoparasites often lived on the surface of their body cover, ie on the skin, under the skin or in the upper layers of the epidermis (Zajac et al. 2011; Prantlová Rašková and Wagnerová 2013).

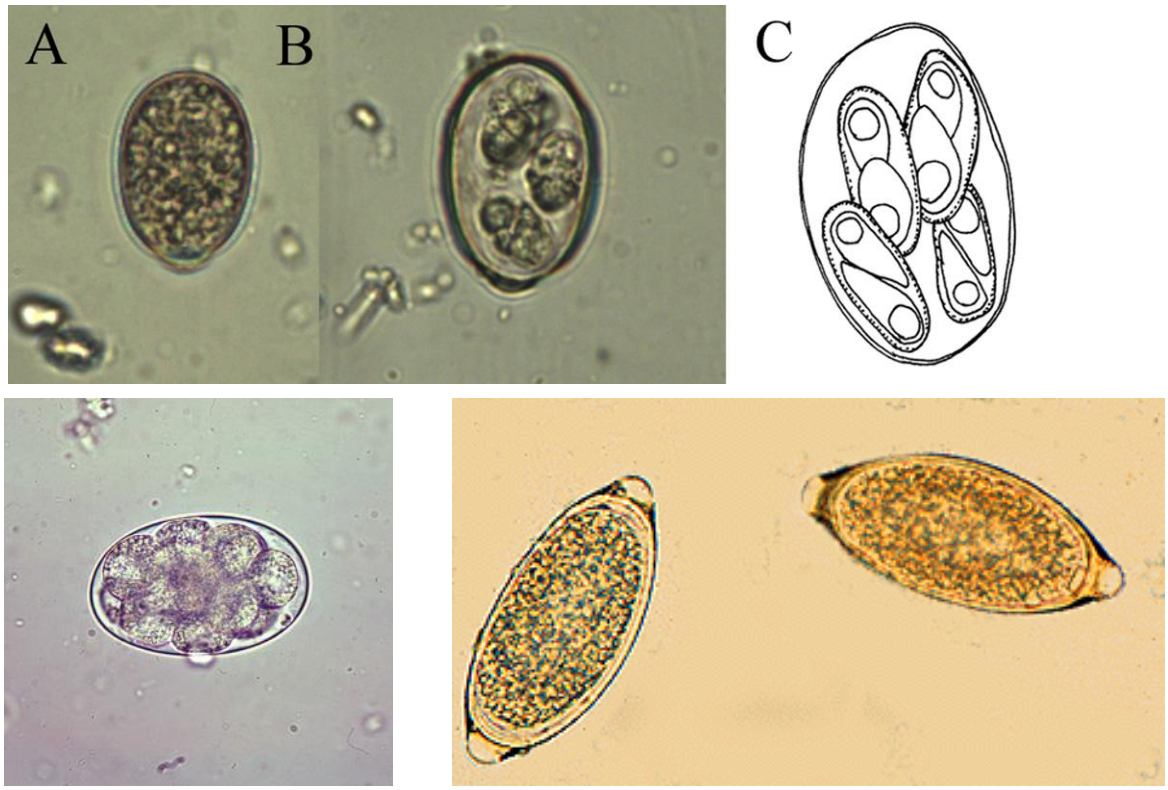

Figure 6: Oocysts of coccidia *Eimeria* spp. and an egg of a nematode of the genus *Trichuris* spp. (Daniela Lukešová)

1.2.3.1. Kokcidie rodu Eimeria

The genus Eimeria belonged to the Apicomplexa and caused a worldwide known disease in young individuals, so-called coccidiosis, in which the intestinal tract of domestic or wild ruminants was infected, as described by Rommel et al. (2000). Developmental stages of coccidia can be observed in faeces in about 3-5 days, so-called oocysts with four sporocysts, and in each of them there were two sporozoites, which after ingestion by the host subsequently infected the intestinal cells. Further development took place in the intestinal cells first asexually (sporogonia, merogony) and was followed by the sexual phase (gametogony) and the production of coccidial oocysts (see Figure 7 – life cycle). The faeces excreted these developmental stages from the host (Daugschies and Najdrowski 2005), when in favorable environmental conditions the oocysts sporulated and were able to survive for a long time.

In sheep and goats, many species of coccidia have been parasitic, eg *Eimeria arloingi*, *Eimeria ovinoidalis*, *E. crandallis*, *E. granulosa*, *E. faurei*. Coccidiosis was caused by infection with food and water, or by licking animals together. The ingested oocysts penetrated into the cells of the intestinal mucosal epithelium (Haták et al. 2008).

Most species of coccidia of the genus Eimeria caused the so-called subclinical coccidiosis, ie diarrheal disease in young individuals. Highly pathogenic species included *Eimeria arloingi* and *Eimeria ovinoidalis*, which caused serious clinical diseases with symptoms of so-called hemorrhagic diarrhea, and especially in young, intestinal lesions, cachexia and exhaustion could occur, often with fatal consequences. The acute form was manifested by high fevers (+40 °C), diarrhea with mucus and blood, as well as loss of appetite, apathy and weight loss, often with colic pain. The chronic form manifested itself in the onset of convulsions, watery diarrhea with blood and mucus, which was later replaced by constipation (Chroust et al., 1998; Najdrowski, 2005; Kváč et al. 2006, Vadlejch 2013).

The prevalence of coccidiosis in sheep and goats was relatively high, it could reach 100%. The most susceptible were mainly young animals aged three weeks to six months (Taylor and Catchpole 1994). The presence of oocysts *Eimeria* spp. can be diagnosed by simple flotation tests or microscopic examination of the intestines during necropsy. Preventive measures consisted of the so-called early diagnosis of oocysts in

animal faeces and oral administration of effective anticoccidials administered preventively (Jagoš 1985).

Sheep coccidiosis has been reported in Europe, America, Australia and Africa and has always been widespread worldwide. In the Netherland Antilles, the prevalence of the clinical form of coccidiosis of small ruminants was determined and the presence of *Eimeria* spp. in faeces in 49.1% of sheep and 34.5% of goats, on the Dutch island of St. Eustatius (Ruitenbeek 2016). Studies in the Netherlands also revealed an 82% prevalence of goat coccidiosis and a 78% prevalence of sheep coccidiosis (Borgsteede 1996). In the United States, 69% of apparently healthy sheep were found to have a positive finding of coccidial oocysts in their faeces (Kahan 2013). In Brazil, in the studies of Macedo et al. (2019), oocyst infection was found in 73.91% in goats and in 66.18% in sheep. In Mexico, the occurrence of *E. faurei* species was 58.64%, then *E. crandallis* in 57.57%, *E. grunulosa* in 56.29% and *E. ovinoidalis* 55.29%. In goats it was recorded in 49.90%, with the most widespread species *E. caprovina* 61.98%, then *E. christenseni* 59.91%, *E. hirci* 59.75% and *E. arloingi* 58.11% (Alcala -Canto 2020).

Their oocysts had 10 to 60μm in length, rarely more. Each oocyst had a protoplasmic mass when passed in the feces. Outside the body, it develops (sporulates) so that it contains four sporocysts, each containing two sporozoites (Thienpon 1986).

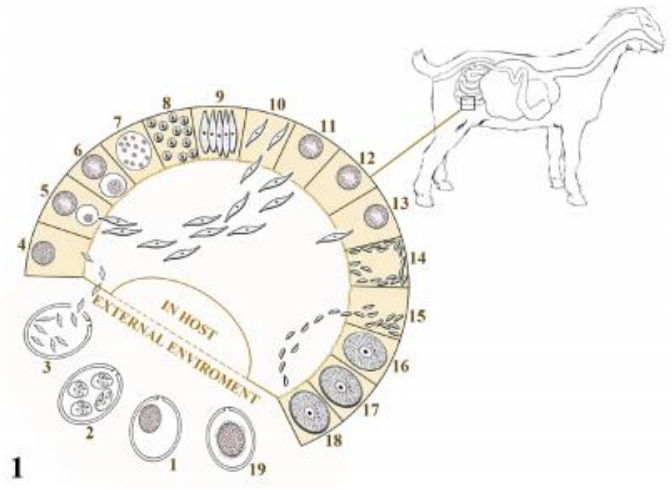

FIGURE 1: Life cycle of Eimeria (diagrammatic). (1) Oocyst; (2) Sporulated oocyst; (3) Liberation of sporozoites; (4) Sporozoites entering epithelial cells; (5-11) Schizogony: formation of schizonts and merozoites; (12) Sporogony: formation of macrogametocyte; (13-15) Sporogony: formation of microgametocytes; (16-17) Development of microgametocyte; (18) Fertilization; and (19) Formation of oocyst [33].

Figure 7: Life cycle of *Eimeria* spp. (revmedvet)

1.2.3.2. Tapeworms of the genus Moniezia

Tapeworms were included in the Cestoda class and their definitive hosts were ruminants and intermediate hosts of mites from the Oribatidae family. Outgoing tapeworm cells from the host were eaten as organic matter by mites, and an infectious larval stage of the so-called metacestod, called a cysticercoid, formed in their body cavity. The formation of cysticercoids took about 15-18 weeks. Thereafter, the mite could be ingested by the final host, where further development took place in the small intestine of the host, until the tapeworm reached sexual maturity (Jankovská, 2012). In ruminants, including sheep and goats, the species *Moniezia expansa*, *Moniezia benedeni*, *Moniezia autumnalis*, *Moniezia baeri*.

The spread of tapeworms in pastures was very common (Dever et al. 2015), in the case of a massive infection there were clinical signs of diarrhea (Gomez-Puerta et al. 2008). In the laboratory, the presence of the Moniezia agent can be determined macroscopically, by observing body segments on the faecal surface, by flotation procedures or autopsy and examination of the small intestine contents of the animal (Jankovská, 2013; Vadlejch et al. 2013).

1.2.3.3. Nematodes of the genus Nematodirus

The parasites were included in the Nematoda (Langrová et al. 2012) and these individuals in the adult form can be seen in the small intestine of animals. The female worms laid the eggs in the intestinal contents, and gradually embryogenesis gradually took place inside the eggs, leaving them in the faeces of their host. Furthermore, in the external environment (pasture, stable) the larval stages L_1 and L_2 developed into the infectious stage L_3, which was resistant to cold and drought in the external environment. In ruminants, including sheep and goats, nematodirus species are parasitic: *Nematodirus abnormalis, Nematodirs battus, Nematodirus spathiger*, *Nematodirus filicollis*.

The relatively high pathogenicity was caused by the species *Nematodirus battus* and *Nematodirus spathiger* in animals, when clinical symptoms (diarrhea and dehydration) occurred. All types of nematodes can be diagnosed after flotation of faeces samples and subsequent microscopic examination (Prantlová Rašková and Wagnerová 2013).

It was 10 to 30 mm in length in the adult stage. The eggs were very large and distinctive. They were 180-230 by 90-107 μm, smooth and elongate, tapering at both ends. The eggs were segmented (eight cells) when paired in the feces. Infective larvae, which were 900-1,100 μm in length, developed in the eggs in 2-4 weeks. The infective eggs may survive over winter (Thienpon 1986).

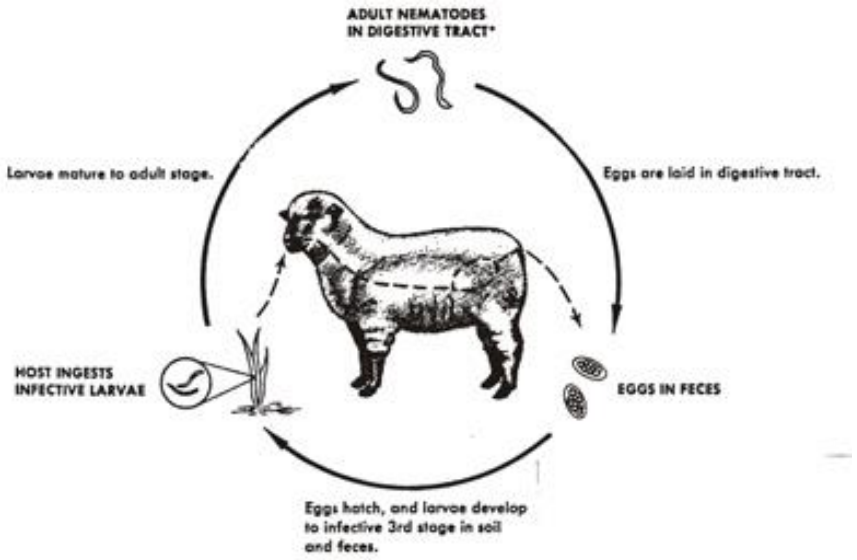

Figure 8: General life cycle of gastrointestinal worm parasites (internal parasites)

1.2.3.4. Nematodes of the genus Trichostrongylus

Genus of Trichostrongylus and species *Trichostrongylus colubriformis, Trichostrongylus vitrinus, Trichostrongylus rugatus* were usually found in the digestive tract, especially in the small intestine, of domesticated and wild herbivorous animals. The infection could occur after accidental ingestion of vegetatively contaminated third- degree L3 infectious larvae. *Trichostrongylus orientalis* has been reported rarely in humans, although it has predominantly infected various species of animals (Beaver et al. 1984).

The WHO (2002) stated that nematode control programs were aimed at preventing the transmission of infectious stages and thus death. A number of case studies have shown that regular dehelmintisation in families has reduced chronic morbidity in high-risk groups, such as children and women of childbearing age, and prevented reinfections.

Because of their similarity, the following genera have been grouped under the name "**trichostrongyles of sheep**." Of this group of roundworms, *Haemonchus contortus*, the barberpole worm, was the largest and most distinctive in appearance. It had 10 to 30 mm in length in the adult stage. It got its common name from the white ovaries that curl around the straight, red intestine and also called the large stomach worm. Others of this group included *Cooperia* spp., *Ostertagia circumcincta* and *Trichostrongylus* spp., particularly *Trichostrongylus colubriformis*. They had two to 12 mm in length in the adult stage and were not distinctive macroscopically. *Haemonchus contortus* and *Ostertagia circumcincta* are bloodsuckers. The eggs of all four genera were so similar in appearance that they cannot be differentiated. The eggs were elongate ellipsoidal and smooth, with 80-100 by 40-50 μm. They were segmented (8 to 32 cells) when passed in the feces (Thienpon 1986).

The life cycle of intestinal *Trichostrongylus* spp. was direct. Within 18-21 days, they develop into adults lasted. The main symptoms were anorexia, persistent diarrhea, weight loss and venous atrophy (or stunting of the villi) resulting in impaired digestion and malabsorption, with loss of protein occurring on the damaged mucosa (Fox 2014).

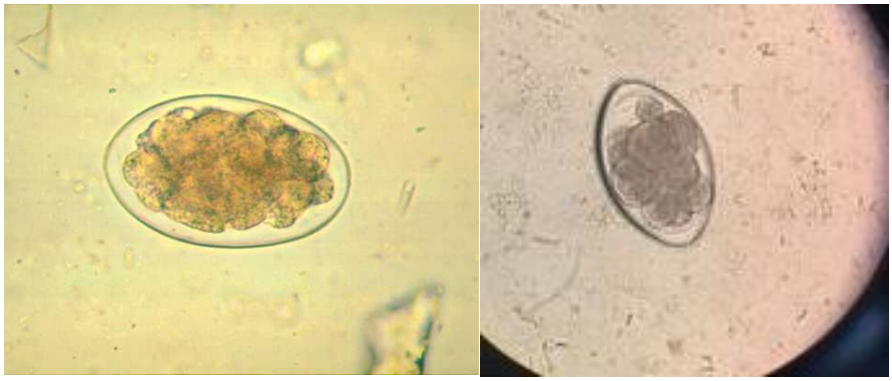

Figure 9: Eggs of *Trichostrongylus* spp. and *Nematodirus* spp. (Dominika Roudná)

1.2.3.5. Nematodes of the genus Cooperia

The genus Cooperia was included in the Nematode and this species of nematode most often parasitized in ruminants. Their life cycle became typical of the so-called super-family Trichostrongyloidea, with the exception that they often received intestinal contents even with a small amount of host blood. After ingesting infectious larvae (on pasture or in a stable), the larvae were attached to the mucosa of the small intestine, where they damaged tissues and vessels, and grew to adulthood to produce eggs when they reached adulthood, from which larval stages hatched (Zajac et al. 2011). In sheep and goats, the species *Cooperia curticei*, *Cooperia surnabada*. Species *Cooperia* spp. were considered to be mildly pathogenic organisms (Junquera 2021).

The main clinical signs of infection were mostly diarrhea, dehydration and lower body weight. Eggs of the species *Cooperia* spp. could be demonstrated ovoscopically, using flotation methods, while hatched larvae and their morphological features were demonstrated by culturing so-called coprocultures and adult nematodes detected by small intestinal autopsy (Hofírek et al. 2009). Cooperia larvae were very resistant to adverse environmental conditions and able to survive up to 12 months, or survive the winter as so-called dormant larvae, which made it difficult to reduce their population. The animals were able to gradually develop a natural way to protect their health, with the ability to spontaneously eliminate parasites from their body. Such resistant animals did not become ill, but continued to shed eggs, which unfortunately could contaminate the external environment (Junquera 2017).

1.2.3.6. Nematodes of the genus Ostertagia

Nematodes of the genus Ostertagia were included in the Nematoda and the species *Ostertagia ostertagi*, often infecting sheep, goats and other domestic and wild ruminants. The parasite did not need any intermediate hosts to survive, the so-called geohelmint. Adult females laid eggs in the stomach or intestine of the host, which was excreted in the faeces of the host organism. Infectious larvae hatched from them, which were able to survive on pasture for up to 14 months. Hosts became infected during grazing with infectious larvae on pastures (Zajac et al. 2011).

Two types of disease have been described: type I ostertagiosis, which affected a young population during the first grazing period when they first became infected. Furthermore, type II ostertagiosis, which affected mainly the adult population, when the larvae resumed their development in winter and spring. The parasites invaded the stomach wall, where they burrowed and proliferated. However, the cells were unable to produce acid to digest food, resulting in an increase in pH in the abomas. This led to the inability to transform pepsinogen to pepsin and prevented protein denaturation and subsequent digestion in the gut. The clinical signs of infection with *Ostertagia* spp. often included diarrhea, loss of appetite, gastroenteritis, dehydration. Massive infection could lead to high mortality. Eggs of the species *Ostertagia* spp. can be detected by flotation techniques and in adults by subsequent autopsy of abomas. The diagnosis can be confirmed by measuring the pH of the rumen fluid in animals (Prantlová Rašková and Wagnerová 2013).

Adult worms were brownish to 12 mm long and relatively thin. The body was covered with a flexible but relatively rigid cuticle, with several longitudinal ridges. The egg was asymmetric, 45x85 micrometers, and had a thin shell, containing 16 to 32 cells (blastomer). Their life cycle was straightforward (parasitipedia.net 2017).

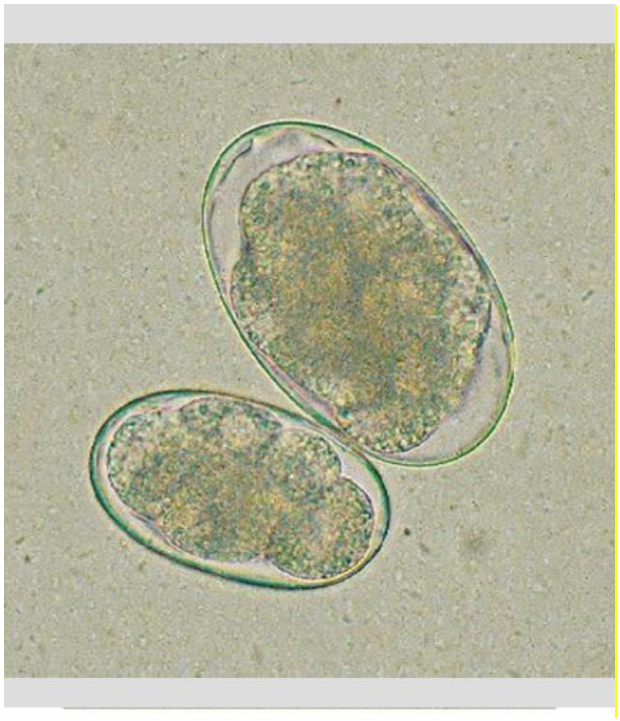

Figure 10: *Ostertagia* spp. in the sheep (Dominika Roudná)

1.2.3.7. Nematodes of the genus Strongyloides

According to Kassai (1999), *Strongyloides papillosus* were classified among the so-called parthenogenic females, present in the small intestine of sheep, among villi (finger-like protrusions of the wall lining). The infection occurred after ingestion of infectious larvae (stage L_3) orally, with food, water (passive infection) or percutaneous (active infection) by infected larvae of stage L_3. Furthermore, there was the possibility of so-called galactogenic infections, ie larvae that migrated to the mammary gland through the bloodstream, just before birth (Šibalić and Cvetković 1996). Migratory larvae and adult forms mechanically damaged host tissues and secreted secretory products. In young individuals, *Strongyloides* spp. sudden death syndrome due to heart failure (Abott and Lewis 2005).

Strongyloides papillosus, the threadworm of sheep, was a long, slender roundworm. These worms were 3.5 to 6 mm by 50 to 65 μm in the adult stage. The eggs were ellipsoidal, smooth and thin-walled. They were 40 to 64 by 20 to 42 μm and the eggs were embryonated. On vegetation or in the soil, the larvae develop to the infective stage (homogonic cycle) in about two days. Some develop into free-living adults, which produce infective larvae (heterogonic cycle) about four days after the eggs were passed in the feces. The free-living females were 1 to 1.5 mm in length. The infective larvae had a long, straight esophagus about half the length of the body. They were 575 to 640 by 16 μm. Infections with these worms were usually diagnosed by identifying the eggs found in the feces. Diagnosis can also be made at autopsy by identifying the worms found in scrapings of the intestinal mucosa (Thienpon 1986).

1.2.3.8. Nematodes of the genus Trichuris

Trichuris globulosa, *Trichuris discolor* and *Trichuris ovis* were included among the species of parasites that infected the most in sheep and goats. These nematodes were characterized by the anterior hair part, which represented two thirds of the body length (the total body length according to the species is 40-75 mm) and a thick back part, where the reproductive and other organs were placed. They had a monoxenic developmental cycle, in which the female laid a large amount (up to several thousand eggs a day) for two years. The development lasted four weeks in stable conditions and even eight to 16 weeks

in the paddock and pasture. In the definitive host, the larvae left their eggs, where they undressed four times in the appendix and colon, and the adults burrowed their hair into the mucosa, where they ate blood. The patent period lasted 50-55 days.

Adults caused juvenile bleeding in the intestinal mucosa by their permanent movement in the intestinal mucosa, and inflammatory to diphtheroid changes occurred due to secondary infection, which could spread throughout the appendix during severe infections.

Only the young population showed clinical signs, the elderly did not manifest symptoms. Dehydration, weight loss, anemia and diarrhea took place, when adult worms could also appear (Hofírek et al. 2009). The disease can be cured if the presence of eggs has been confirmed by the flotation method (Kváč et al. 2001).

1.2.4. Occurrence of gastrointestinal nematodes (GIN)

Parasitosis of small ruminants from the Netherland Antilles, on the Dutch island of St. Eustatius, occurred in the genus *Strongylu*s spp. with 82.7% in sheep and 91.0% in goats. According to Ruitenbeek (2016), other important nematode species have been described on the island, such as *Strongyloides papillosus* (19.2% in sheep, 22.1% in goats), tapeworm eggs (7.6% in sheep, 12.1% in goats) and to a lesser extent eggs of nematodes of the species *Trichuris ovis* (5.1% in sheep, 9.7% in goats). In Mexico, sheep were found to be 85.5% and goats 88.9% (Olivas-Salazar 2018). Lins (2019) described an incidence of 84.7% in sheep in Brazil, and nematodes of *Trichostrongylus* spp. were diagnosed among the most common parasitic species. (13.8%), *Oesophagostomum* spp. (3.6%) and *Strongyloides* spp. (2.4%).

1.2.5. Interaction between parasite and host

The interaction between the host and the parasite has usually been applied mainly to those parasitic groups where the species present either coexisted in a way that could be of benefit to them or detrimental to one or more of the species involved (Randhawa 2012).

Sheep and goats were infected with a number of gastrointestinal parasites, which caused similar pathological changes, with consequent economic consequences in animal

husbandry. Most of the information regarding the interaction between the host and the parasite was obtained from scientific studies focused on sheep, so that they could later be applied to goats. It can be assumed that due to different evolutionary developments, sheep and goats had two different strategies for regulating gastrointestinal parasite infections, based on the immune response and feeding behavior (Hoste et al. 2008).

These two different strategies were based on a balance between the development of an immune response (in sheep) or the existence of a behavioral response that limited contact with infectious larvae (L_3 stage) present in the environment (in goats).

Goats were thought to avoid grazing L_3 larval infection when grazing, where the larva migrated to the tops of grasses, where it was eaten and then traveled to the gastrointestinal tract, where it developed to the L_4 stage. Further studies could contribute to the understanding of these interactions (Hoste et al. 2010).

Differences in animal responses to the gastrointestinal nematode in sheep and goats were first described in the grazing of these species, eg in Australia or Scotland, where a higher degree of parasitic infection was demonstrated in goats compared to sheep. These results supported the hypothesis of a lower degree of immune response in grazing goats to helminth infections compared to sheep (Hoste et al. 2008).

Complete responses occurred in goats on average after twelve months, while in sheep after half a year (Vlassoff et al. 1999). In addition, in breeding goats, the level of infection was almost the same in adults and young animals, in contrast to sheep, where the manifestations of infection in adult females were less severe (Hoste et al. 2008). Another difference was the excretion and accumulation of parasitic eggs in the animal´s body. Adult sheep intensively excreted eggs from the body in larger amounts, while goats rather accumulated them inside the gastrointestinal tract and continuously excreted in smaller amounts (Huntley et al. 1995).

1.2.5.1. Comparison of grazing behavior of sheep and goats

The main difference between sheep and goats was in their feeding behavior. Sheep, as a grazing animal, permanently grazed the ground layers of the stand, while goats tended to graze and graze the middle and terminal parts of plants, shrubs and trees (Smith et Sherman 1994).

From an evolutionary point of view, this significant difference in behavior led to increased contact with a number of plant secondary metabolites, some of which were considered toxic, and thus reduced contact with infectious larvae (L_3). These differences were thought to explain the remarkable ability of goats to detoxify exogenous chemicals, including anthelmintics, and this reduced ability to elicit an effective response against gastrointestinal parasites (Hoste et al. 2011).

Sheep preferred the use of nutrients by the immune system and deprived themselves of the nutrients necessary to maintain resistance to parasites. On the contrary, research focused on the feeding behavior of goats reported that goats did not rely on eliciting an immune response, but strategically used other options, such as less nutrients ingested to increase resistance and immunity, and their organism could better balance (Hoste et al. 2008).

1.2.5.2. Influence of climatic conditions during goat grazing on the occurrence of GIN

The climate in the area has always determined the presence of parasitic agents and their life cycle. The parasites were able to adapt to different environmental conditions and developed their own strategies. The temperature affected the rate of development and survival of the wild stages of the parasites, where moisture prevented the larvae from drying out and allowed the larvae to migrate to the vegetation. Vegetation density and height also affected parasite transmission. Dense and flowing grasses provided a moist and cooler microclimate, thus protecting the larvae from sunlight and drying out.

Parasitic GINs were present around the world, almost from the poles to the equator, and it was impossible to briefly describe the dynamics of infections across different latitudes. However, it was worth exploring some general features of helminth biology and infection dynamics (Torres-Acosta et Hoste 2008).

Adult parasites produced eggs within the host´s digestive tract that were excreted in the faeces of pasture (contamination), where they developed into infectious L_3 larvae over time. These larvae then migrated vertically to the grass, where they were eaten by a grazing animal and subsequently developed into an adult parasite in its digestive tract. Reducing the infectious burden of GIN grazing animals can be considered as disrupting this cycle by controlling the sources of infection, thereby reducing pasture contamination (Houdijk et al. 2012).

These L_3 stages were responsible for infections of pasture hosts and posed a major risk to the animals, being very resistant to external factors, both chemical and physical. L_3 survival was affected by climatic conditions, as in tropical / subtropical regions their viability was relatively short, about one to three months. In mild conditions, depending on the type of nematode, the average survival time L_3 was longer, ranging from six to 12/18 months. The third developmental stage was prone to prolonged drought and frost. The design and implementation of some control measures, especially those that depended on grazing management, relied heavily on knowledge of the relationship between the parasite and the environment (Torres-Acosta et Hoste 2008).

Humid weather in spring and summer has been associated with an increased annual incidence of GIN in goats; seasonal meteorological maps should be used when designing GIN control programs in goats, based on annual records (Valentine et al. 2007).

Typically, 50% of L_3 larvae were found on the first 2 cm of common grasses, while less than 5% were found at 5 cm and above, suggesting that the number of L_3 present on other parts of the plants depended on its morphological structure. For example, the presence of epidermal growths, such as trichomes on the stems and leaves of various types of grasslands or over a larger area of leaves, could have prevented the vertical migration of larvae on the stand. As a result, hosts grazing at the same height could grow fewer parasites and thus successfully counteract the reduction in GIN infection (Houdijk et al. 2012).

A notable exception was *Strongyloides papillosus*. Repeated research showed that the third stage of the infectious L_3 larva did not survive in hay or silage, which explained why breeding conditions inside zero-grazed stables were unfavorable for GIN development (Torres-Acosta et Hoste 2008).

The larvae of *Strongyloides papillosus* penetrated intact skin, entered their capillaries in sheep and goats and traveled through blood to the lungs, through the trachea to the digestive tract and intestines. No histological changes in the skin were observed after the first exposure, but pustular dermatitis developed after repeated reinfections (Smith et Sherman 1994).

1.2.5.3. Grazing management

Methods based on grazing management strategies have been described since the end of 1960, and their use was subsequently evaluated under different climatic or epidemiological conditions. The general aim of these methods was to provide susceptible animals with pastures with minimal biological burden. This goal could be achieved through different grazing management options in different ways (Torres- Acosta et Hoste 2008).

Dispersion was important for all species, as the population, which would be spatially limited to a small area, was at risk of extinction in the event of adverse conditions. Dispersion reduced the possibility of inbreeding and the loss of evolutionary adaptability. Three aspects of propagation have been described: scattering over short distances from individual hosts, scattering in space and the extent of spreading over longer distances, and finally scattering over time (Rohde 2001).

The use of rotary grazing has added another dimension in controlling the spread of GIN. The longer the pasture remained fallow, the lower the load on the larvae. If we treated animals before starting a rotational grazing system, we should be able to control parasitism (Stromberg et Averbeck 1999).

According to Hoste et Torres-Accosta (2011), they described that farmers and advisers should not consider the control of helminthosis as a top priority in rotary grazing, which could lead to a negative impact on productivity and animal health. Due to the persisting differences in the survival of L_3 dominant species in temperate and tropical climates, rotational grazing seemed to be a suitable choice for GIN control only in hot and humid climates, with *Haemonchus contortus* and *Trichostrongylus colubriformis* larvae dying after only four weeks. The efficiency of rotational grazing was questionable in mild climatic conditions, when the larvae of *Trichostrongylus* spp. could survive 6-12

months. In all cases, the rotary grazing system should be designed to make the best use of fodder on the pasture (Hoste et Torres-Accosta 2011).

Barger (1999) stated that two or more host species in a given environment did not share common parasite species and that interspecies rotation could be a successful means of increasing GIN control. Small ruminants and cattle, small ruminants and horses or horses and cattle seemed to be logical candidates for an alternative grazing strategy as long as *Trichostrongylus axei*, which could infect all these host species, did not become a major problem. The rotation of sheep and goats on pasture was probably not successful due to the fact that they were infected with the same species of parasites.

Cattle were infected with the same species of parasites as small ruminants, but usually had their own strains. In general, nematode species of small ruminants have unsuccessfully infected horses or cattle, and larvae have died after ingestion by these host species. However, two exceptions should be noted. Calves could be susceptible to *Haemonchus contortus* and should not be exposed to pastures contaminated with this parasite, and *Trichostrongylus axei* had a wider host specificity and successfully infected both cattle and horses (Zajac 2006).

1.2.6. Immunity and its effect on the occurrence in the gastrointestinal tract

1.2.6.1. Immune system reactions

Immunity has become the host´s main defense mechanism. After the infectious agents have entered the host body, the immune system has always responded, mobilizing various components, such as lymphocytes, eosinophils, etc., that have invaded and eliminated the invaders (Miller et Horohov 2006).

Defensive responses against parasites at the humoral and tissue levels were based on the host´s ability to distinguish its own cells from foreign ones. Vertebrates had three types of such reactions: phagocytosis, inflammation, and adaptive immunity. All of these defense mechanisms usually occurred in most tissues and organs. Typically, these reactions occurred in a certain order: degeneration or necrosis of the cells due to infection, leading to an inflammatory reaction and edema. Phagocytic cells absorbed small

parasites, but if the parasites were not excluded, chronic inflammation developed, leading to the formation of a capsule in the connective tissue around the parasite. Macrophages in the capsule absorbed damaged cells and often parasites. Immune responses have been elicited by parasite antigens that have led to the production of specific antibodies in the host (Rohde 2001).

Meeusen et al. (2005) stated in their study of an innate immune response to the presence of gastrointestinal nematodes parasitizing in the digestive tract of ruminants (specifically *Haemonchus contortus*) that the economic severity of nematode infections increased with the increasing resistance of these parasites to anthelmintics.

The main mechanism by which animals acquired immunity against parasites became recurrent infection, with effector mechanisms of the ruminant immune system being able to adapt to the different life cycles of individual parasites. The study focused on the identification of parasitic antigens and molecular components of the ruminant immune system that were involved in the elimination of these antigens (Meeusen et al. 2005).

The process of elimination of the pathogen by the immune system was initiated by the recognition of parasitic antigens, the induction of an appropriate phenotypic immune response, the activation of effector cascades and the final expulsion of the parasite. This final destruction of the parasite in gastrointestinal parasites has been associated with the action of specific antibodies, unique effector cells and T2 cytokines (Meeusen et al. 2005).

According to Meeusen et al. (2005) made the isolation of specific antibodies against parasites complicated by the fact that gastrointestinal parasites within the host body moved very quickly from one larval stage to another. This problem in their study by Meeusen et al. (2005) solved by isolating local antibodies produced by antibodies by so-called secreting cells (ASCs) in local lymph nodes at a time when the current larval stage was reliably identified. By this procedure, so-called ASC probes were obtained, thanks to which it was possible to isolate L_3 specific antigen (HscL3) expressed on the surface of L_3 larvae. Vaccination with purified HscL3 antigen resulted in a significant reduction in parasitic infection of infected sheep.

1.2.7. Biosecurity

Biosafety has been defined by the FAO as "Implementing measures to reduce the risk of outbreaks and the spread of disease" (FAO 2010). In the European Union, the emphasis on biosafety is a new motto: "Prevention is better than cure" (European Commission 2007).

Biosecurity measures prevent the direct transmission of animal pathogens as well as indirect transmission between farm animals, as described by Ellis-Iversen et al. (2011). According to Lin et al. (2003), the prevention of animal diseases has become an important measure that can lead to the reduction or even elimination of therapy in farms. According to Sarrazin et al. (2014) The implementation of biosecurity included all prevention measures, from the entry of pathogens into the herd (external biosecurity), which led to a reduction in the spread of pathogens in the herd (internal biosecurity).

In livestock farming, biosecurity was directly dependent on breeding technologies, where rearing livestock on pastures allowed input costs to be reduced during the grazing period and often allowed for improved animal welfare (Waller 2006).

Basic biosecurity measures, on the other hand, were better applied when animals were housed in an enclosed area and reared on a rotating basis. Adherence to these measures may have been complicated by different housing technologies than in pasture animals (Stromberg and Averbeck 1999).

Biosafety has been affected by a number of factors. In the case of mutual contact of a large number of animals, the inclusion of new animals in the herd was a significant risk. Preventive measures included a thorough health inspection of the animals before purchase and transport and obtaining essential information on the origin of the animals, the health status of the herd and the epidemiological situation in the region where the purchased animals came from. All transport vehicles should be cleaned and areas disinfected with effective chemicals before the animals are loaded and unloaded. The health of the animals on the pastures had to be checked daily and all unusual manifestations of the animals' behavior should be recorded in their medical records (strange behavior, sudden deaths, increased number of sick animals). At the same time, the measures should apply not only to the transport of animals, but also to the origin of

feed and water resources intended for feeding the herd (Novák et al., 2017; Novák et al. 2012).

The human factor played an important role, as the care staff should follow basic hygienic procedures during contact with the animals. During the grazing period, the entry of unauthorized persons into the pasture should be a problem for cattle, if hiking or cycling trails pass through them. Similarly, the entry of domestic or wild animals (dogs, cats, game, etc.) should be prevented.

The ecological factor included mainly climatic parameters, mainly temperature, rain, humidity, air flow, sunlight. Grazing conditions became a suitable place for the survival of the developmental stages of helminths, especially eggs, and the subsequent development of larval stages, when the host organism was infected after ingestion of infectious larvae (Stromberg, 1997; Stromberg and Averbeck 1999; Waller 2006; Sahlstrom et al. 2013; Sarrazin et al. 2014; O'Mahony 2015).

One way to increase biosecurity was effective management control, where anthelmintics were collected and administered at the appropriate time, in collaboration with veterinarians, to ensure biological control of parasites causing eg. coccidiosis and helminthosis in animals.

The occurrence and process of parasite infection was influenced by several factories (age of animals, types of breeding, climatic conditions, etc.). In the past, excessive use of anthelmintics has been a common solution for treating infected animals, leading to an increase in resistant nematode strains, which are now a global problem. The current trend has become targeted treatment or targeted selective treatment of clinically ill animals. Therefore, it was very important to detect possible parasitic infections as soon as possible (Novák et al. 2012; Novák et al. 2017).

1.2.8. Elimination of parasites from the host

Since the beginning of the 20th century, various chemical compounds have been developed for the production of medicinal products against endoparasites, most often based on arsenic, tin and nicotine. These preparations were replaced by phenothiazine in the 1930s, and in the 1960s the first broad-spectrum anthelmintic appeared, effective against various types of parasites, based on thiabendazole, from the group of so-called benzimidazoles. A little later, tetrahydropyrimidines appeared on the market, such as

pyrantel, morantel, oxantel, which are mainly effective against nematodes. Around the same time, another group of anthelmintics, so-called organophosphates, such as dichlorvos, entered the market. Finally, in the 1980s, the first macrocyclic lactone, ivermectin, was launched. At present, there are a number of very effective preparations against gastrointestinal nematodes parasitizing in sheep, classified according to their mechanism of action into several groups.

The group of benzimidazole anthelmintics has become the most extensive group of antiparasitics derived from a single chemical structure. Some benzimidazoles belonged to the anthelmintics with the widest spectrum of action. These drugs were effective on nematodes, partially on trematodes and cestodes, and were relatively non-toxic. Most of the drugs in the group acted against the developmental and adult stages of helminths, some substances had a so-called ovicidal effect (Langrová 2014).

Ivermectin became historically the first drug of the avermectin group, always one of the most important drugs in veterinary medicine. It is the active substance in a preparation commercially called Ivomec® (Boehringer Ingelheim, Ltd.comp., CR), which has been used to treat parasitosis in a wide range of hosts. Doramectin and galmectin also belonged to the group of avermectins, they had similar properties as ivermectin (Abbot et al. 2012).

According to Herd and Coles (1995), avermectins have been described as drugs of biosynthetic origin and the so-called third group of broad-spectrum anthelmintics. This group was very effective against the developmental stages and adults of nematodes. Avermectins acted on the glutamate gate of the chloride ion channel and caused non-spastic paralysis of nematodes.

From a chemical point of view, anticoccidials can be divided into a group of synthetic substances and derivatives, natural substances and biosynthetic substances (so-called ionophores). Synthetic substances, especially sulfonamides (sulfaclosin, sulfadimethoxin, sulfadimidine) acted due to their structural analogy with para-aminobenzoic acid (PABA), which entered the synthesis of folic acid in the coccidial cell, blocked PABA and folic acid metabolism and thus prevented the development of coccidia et al. 2014).

The use of conventional antiparasitics as the only method to control the occurrence of parasites was not possible and new, alternative methods were needed. Plants containing

biologically active substances, especially tannins, have great potential for these methods (Hoste et al. 2006; Torres-Acosta and Hoste 2008). They have shown anthelmintic activity and indirectly acted to increase host resistance to parasite-induced infections (Shaik et al. 2006; Burke et al. 2012). In recent years, great attention has been paid to alternative methods in the fight against parasites in the Czech Republic. Traditional medicinal plants were used, especially ethanol extracts from plants of the species *Allium sativum* (garlic), *Artemisia absinthium* (wormwood), *Consolida regalis* (Urban wormwood) according Urban et al. (2008; 2014).

1.2.8.1.1 Chemical drug – Vecoxan®

Vecoxan® was marketed as an oral suspension with the active substance diclazuril 2.5 mg/ml, a benzoacetonitrile anticoccidial without antimicrobial activity, which was effective against coccid species of *Eimeria* spp. Diclazuril had an effect on the asexual or sexual stages of the parasite's developmental cycle. Treatment with diclazuril had only a limited effect on intestinal lesions caused by parasitic stages older than 16 days. Diclazuril treatment resulted in interruption of the coccidial cycle and oocyst excretion for approximately two weeks. This allowed the animal to bridge the time of decline in maternal immunity (observed at approximately four weeks of age). The absorption of diclazuril in lambs after oral administration of the suspension was low. Maximum plasma concentrations were reached approximately 24 hours after dosing. Absorption decreased with age. Frequent and repeated use of antiprotozoals has led to the development of resistance in these parasites. In addition to diclazuril, additional supportive treatment was required to improve the course of the disease, in the case of confirmed clinical coccidiosis in individual animals already showing symptoms of diarrhea, as diclazuril had no evidence of antimicrobial effects.

1.2.8.1.2 Natural preparation – Emanox®

Emanox® has been used to prevent and treat coccidiosis in all species of animals, especially rabbits, poultry and small ruminants - calves, lambs and kids. It was made from extracts of medicinal plants *Origanum vulgare* (oregano), *Thymus* (motherwort), *Mentha* (mint), *Salvia rosmarinus* (rosemary), *Origanum majorana* (marjoram), *Allium sativum*

(garlic) and others. Due to its varied composition (mixture It has been described as a natural product and can be used without restriction (withdrawal period) in the production of organic products, regardless of the time of slaughter of the animals. Emanox® was not classified as a medicinal product and was therefore not subject to medication and feed regulations and was not one of the substances monitored for residues and contamination. Universal product for the prevention and treatment of coccidiosis in all species and categories of animals.

1.2.8.2. Resistance

Long-term use of antiparasitics has developed selection pressure for parasites, which has caused selected individuals to become resistant to the selected substance, and this problem mainly concerned the breeding of herbivores. In some areas, such as South Africa, Latin America and Australia, parasite resistance to substance use has reached a point where sheep and goat farming in the region has been unsustainable under current conditions (Abbot et al. 2012) and resistance to all hitherto known broad-spectrum anthelmintics (Wolstenholme et al. 2004) and the so-called multi-resistance also developed, when nematodes were resistant to several active substances at once (Cezar et al. 2010).

Clinical resistance occurred when the selection pressure was high and the resistance alleles exceeded a certain level (Sangster and Dobson 2002). The most important rule was to preserve the so-called refuges (parasitic population), which became increasingly susceptible to common deworming agents. Also during the so-called overuse of anthelmintics, the "refuge" was eliminated rather than the creation of a "resistant population", which accelerated the onset of resistance (Bath 2014). To avoid accelerating clinical resistance, it was necessary to avoid frequent use of the same drug throughout the year and to adhere to the recommended dose, administered to all animals in the herd at once (Fleming et al. 2006; Abbot et al. 2012).

1.2.8.3. Caribbean import requirements for animals with increased antiparasitic resistance

Anthelmintic resistance has become a widely recognized problem in many parasite populations and could have a negative impact on animal health, animal welfare and livestock production potential (Kaplan 2004; Schnyder et al. 2005; Crook et al. 2016).

Anthelmintic resistance has increased over time (Kaplan 2004), and in some areas of the United States, resistance to ivermectin and benzimidazoles has developed in the ruminant herd, ranging from 80 to 100% (Crook et al. 2016).

When the animals were imported, all the animals, I quote: 'were treated with an anthelmintic and flukicide to control internal parasites, including liver fluke, within fourteen days of dispatch'. The veterinarian administered Ivomec Plus® subcutaneous preparation to each animal. Upon arrival, the animals were quarantined. The development and improvement of small ruminant farming in the Caribbean is a priority for the Food and Animal Organization (FAO) in order to improve food security in these developing countries (FAO 2020b).

1.3. Flotation methods

Flotation-centrifugation techniques have been commonly used to diagnose the developmental stages of gastrointestinal (GIT) parasites. These non-invasive, relatively inexpensive methods could detect the presence of parasites in the host organism. Parasites living in the intestinal tract of animals always secreted oocysts, cysts or eggs that left the host's body through the faeces into the external environment and could be subsequently identified and quantified (Chroust et al. 1998).

Egg counting techniques were preventively recommended to monitor the health of individual species of animals or herds and were used to determine the degree of infection of specific species of parasites. The result of the laboratory examination made it possible to ensure targeted therapy of antiparasitics in the herd, with the help of effective medicinal products.

A positive finding of parasitic stages, on microscopic examination, indicated that the animal was infected, but the degree of infection and clinical condition of the animal could be influenced by a number of other factors (more or less pathogenic species of parasites, individual immune status of the host organism, degree of infection, ie. weak, medium high, massive, etc.), which could lead to a reduction in the number of oocysts or eggs in the faeces of animals, due to different biological cycles of parasites. There was often an irregular excretion of parasitic stages, depending on the season, different climatic environment, etc.

Thanks to the knowledge of the morphology of the present oocysts and eggs, it is possible to recognize individual genera of parasites during the so-called qualitative examination. To subsequently confirm the species representation of GIN parasites in the host organism, it was often necessary to use diagnostic keys and morphological atlases (Zajac et al. 2011).

The numbers of excreted oocysts and eggs of individual parasite species (quantitative examination) could be different. It will always depend on the biological potential and pathogenicity of individual parasitic species (Lukešová 1990; FAO 2015).

Some diagnostic methods may have been characterized by low sensitivity, and thus a low degree of parasitic infection may not have been demonstrated at all.

1.3.1. Principle and procedure of flotation-centrifugation methods

From a practical point of view, flotation-centrifugation methods are used to monitor parasitic infections in animals. Their principle was based on the use of flotation solutions with a higher specific weight than the weight of parasitic oocysts or eggs, generally speaking, the higher the density of the flotation solution, the higher the possibility of capturing parasitic developmental stages floating in the surface blank.

Laboratory procedures could in many cases be different, depending on the conditions of the laboratory equipment. A simple procedure suitable for field conditions was to mix a small amount of faeces directly with the flotation solution in a graduated cylinder or tube, leaving until the bottom of the cylinder became clear and the contents rose to the surface of the cylinder (Dryden et al. 2005). The surface blank can be transferred from the surface using a wire loop to a microscopic slide and the sample was examined using a light microscope.

A modification could be to fill the measuring cylinder or tube completely to the edge of the cylinder or tube and to apply a glass cover glass (Zajac et al. 2011). After a long time, the cover glass was carefully transferred to the slide, followed by microscopic diagnostics.

A number of flotation-centrifugation techniques have been used in common veterinary practice in the conditions of the Czech Republic, eg Breza's, Sheather's method, etc. (Lukešová 1990).

1.3.2. McMaster's method

The traditional method of counting eggs and oocysts in faeces was developed in 1939 in Australia, for easier and routine detection of parasitic stages in sheep faeces (Gorden and Whitlock 1939). There have been modifications of this McMaster method worldwide, with a number of variations, such as different faecal weight, volume and type of flotation solutions, different sample dilutions, flotation time, use of post-centrifugation, duration and rate of post-spin (Vadlejch et al. 2011). The problem of the methodology was insufficient sensitivity, especially with a low number of eggs. According to Coles et al. (1992) McMaster's method was accurate from the detected 50 EPG (egg per gram) in faeces. In some cases, due to modifications, the method was refined with sensitivity up to 10 EPG (Cringoli et al. 2010). Another problem could be the use of very small amounts of animal droppings.

1.3.3. FECPAK^{G2}

The commercial FECPAK^{G2} kit from New Zealand (faecal egg count in package) has essentially become a modified version of the McMaster method, without the need for centrifugation (Coles et al. 2006). The faeces samples were mixed with the flotation solutions and their contents were then pipetted into two chambers. The eggs floated to the surface and their numbers could be recorded in grid cells under a microscope (Goldber et al. 2014). The method was often used in field conditions in horses (Presland et al. 2005) and its use was simpler than the laboratory McMaster method.

1.3.4. FLOTAC

A relatively new method, developed in 2009 by the team of Professor Cringoli of the University of Naples, became the FLOTAC method, which could be used in three different modifications (Cringoli et al. 2010).

1. The basic examination method was designed to determine a very low number of parasitic eggs from one individual. The sensitivity of the basic FLOTAC technique was one EPG (egg per gram) in the faeces.

2. The examination method, the so-called “dual technique”, was based on the use of two different flotation procedures and was used to test the same faeces sample in parallel. The methodological procedure was suitable for the so-called epizootiological

(syn. epidemiological) monitoring and routine diagnosis, in order to perform extensive parasitological screening. The sensitivity of the so-called dual FLOTAC technique was two EPGs (egg per gram) in the faeces.

3. An examination method that used a "dual technique" based on the simultaneous examination of two different faecal samples, from two different hosts, using a single FLOTAC tool. The sensitivity of FLOTAC in the so-called dual FLOTAC technique was two EPGs (egg per gram) in the faeces.

2. Aims of the Thesis

The aim of this diploma thesis was to prove the occurrence of developmental parasitic stages in sheep and goats in selected localities on the island of Aruba. At the same time, information was obtained on chemical and alternative treatment options. Furthermore, laboratory diagnostics, evaluation of the prevalence of parasitosis was performed on the basis of laboratory results and a comparison of the effectiveness of chemical and biological antiparasitics in selected localities.

H1: A higher prevalence of endoparasitosis was found on the island of Aruba compared to the Caribbean island of St. Eustatius.

H2: Classical application of antiparasitics had a higher effect than the use of plant extracts to deworm animals.

3. Methods

3.1. Technology of sheep and goat breeding in Aruba

Sixty Black Headed Doeper sheep and seventy French Apline, Anglo-Nubian and Toggenburg goat were bred on farm A - Santa Rosa - Piedra Plat. One to two males were bred in the herd. The animals were kept in free pens under a shelter with individual farrowing pens and pens for sick animals (quarantine boxes). The animals did not have free access to pasture and were fed hay and compound feed with minerals. The animals were fed in the form of a feeding opening which was freely accessible to each animal. Hygienic and health measures were implemented in the stable. Manure removal was performed daily by the animal breeder. Antiparasitics were administered only to sick individuals.

Blackhead Dorper sheep breeds and Bier goat breeds were bred on farm B - Sero Alejandro - Savaneta. The herd consisted of 90 sheep and 50 goats of all ages. One to two males were bred in the herd. The farm was of the closed type with selected access to pastures. The herd was fed hay and compound feed with minerals. The water source was provided in the form of a feed hole, which was freely accessible to any animal. The animals were kept in free and shared pens under shelter and were divided by species during the night. Due to the danger of attack by wild dogs, it is necessary to protect the animals overnight. The animal keeper removed the feces in the pens every day and once every two weeks on the pasture. No deworming measures were taken on the farm.

Blackhead Dorper sheep breeds and Bier goat breeds were bred on farm C - Sero Grandy - Savaneta, the herd contains all age groups, with one to two males. The herd consisted of 30 sheep and 80 goats. The animals had free access to pasture with a shelter that hid the animals from the adverse climate. The main components of the feed were grazing, hay and compound feed with minerals. The animals were fed in the form of a feeding opening which was freely accessible to any animal. The faeces were not removed. No deworming measures have been taken so far.

Blackhead Dorper and Bier goats were bred on the D - Noord – Jaburibari farm. The herd consisted of 50 sheep and 40 goats of all ages, with one to two males. Individuals were fed hay and compound feed with minerals. Power was provided in the form of a

power port, which was freely accessible to any animal. The farm was of the closed type with a selected approach to grazing. The animals were housed in free and social pens at night and divided by species to protect the animals from wild dogs. Part of the pens formed a shelter, which protected the animals from the adverse climate. Manure removal in pens was carried out daily by the animal breeder and once a week, clearing on pastures was ensured. So far, no deworming measures have been taken here.

Blackhead Dorper Bulgous sheep and goats were bred on the E - San Nicolas farm. The basic herd consisted of 100 sheep and 100 goats, with one to two males. The herd was fed on hay, compound feed with minerals and, above all, grazing. The animals were fed in the form of a feeding opening which was freely accessible to each animal. All age categories and sexes of sheep and goats were kept together, with free access to pastures. Shelter from adverse conditions was natural (trees, forest). So far, no deworming measures have been taken here.

Control sampling of ovine and caprine faeces was performed on all selected farms. Samples were taken in the same number as animal faeces samples to determine the efficacy of the drugs. On Farm B Sero Alejandro (n = 6), C Sero Grandy (n = 5), D Jaburibari (n = 5) and on Farm E San Nicolas (n = 4). Faecal analyzes were performed at the same times, before and after administration of Emanox® and Vecoxan® to selected animals. These untreated animals were of the same age category, i.e. the youngest in the farm, in order to determine the efficacy of the medicinal products.

3.2. Laboratory material

The laboratory used Sheather´s solution, i.e. a solution of sucrose with a specific weight of 1.20-1.30 g/ml, which was higher than the specific weight of the developmental parasitic stages. The faeces samples were recorded in a laboratory protocol and then dispensed, made up of water in plastic beakers, mixed, passed through a gauze and filled into a plastic centrifuge tube, and the sample was provided with a sample serial number. The sequence number of the sample was identical at all times, either on the tubes during subsequent centrifugation and flotation or at the time of reading the results from the native preparation under light microscopy.

3.3. Collection of faecal samples

Samples for coprological parasitological examination to monitor prevalence were taken at two intervals: the first: 20.2. - 23.4. 2019 and the second one: 17.7. - 28.8 2019.

The first sampling was made from five sites, the other two samples from four sites distributed throughout the island of Aruba. Breeding A was located in the Piedra Plat area. Breeds B and C were located in the Savaneta area. Breed D was located in the Noord area and the last breeding E was in the San Nicolas area (see picture).

All breeding farms were privately owned, with the exception of Farm A, which belonged to the Department of Agriculture, Livestock and Fisheries in Aruba. Fecal samples were collected fresh in plastic bags and immediately labeled and stored in a portable refrigerated box. According to the number of animals (10%) in each farm, samples were taken and initially examined as a so-called **mixed sample**.

The third sampling for coprological parasitic examination took place on 15.11. - 15.12.2020. Sampling was performed on four selected farms from the previous monitoring (see above). The initial sampling was carried out on all farms at the same time in order to detect the evidence of parasitic developmental stages in animals in these designated localities and to introduce the application of effective antiparasitic drugs. All samples were taken individually, rectally, from the youngest individuals from sheep and goats.

The following farms were selected for the application of the classical antiparasitic: farm C – Sero Grandy, which was located in the Savaneta area, and farm B – Sero Alejandro, which occurred in the Saveneta area. Due to the fact that it was the application of a registered and commercially manufactured veterinary medicinal product Vecoxan® (Inc. Elanco, UK), a single application was performed, according to the Manufacturer´s instructions. For 2.5 kg body weight, 1 ml of the product, with the active substance diclazuril, was required. Control faecal samples were collected 14 days after administration. Fresh faecal samples were collected into microtene bags, which were labeled and stored in a portable cooling box. Samples were taken individually, from the youngest age categories of animals.

The following farms were selected for the application of the so-called alternative herbal preparation Emanox® ((Biokron Ltd.comp., CR): farm D - Jaburibari in the Noord

area and farm E, which was located in the San Nicolas area. Due to the fact that it was a so-called biological therapy, the application took place daily, for 30 days and Emanox® was administered to the animals in a source of water. The control sample was taken after one month. Fresh faecal samples were collected into microtene bags, which were labelled and stored in a portable cooling box and delivered immediately to the laboratory. These samples were also taken individually, from the youngest individuals.

3.4. Coprological examination of faeces

The pooled sample of the mixture represented 10% of the number of animals from each farm and was thus divided. Mixed and individual faeces samples (5 g) were mixed in a mortar with 10 to 15 ml of water. The resulting mixture was passed through gauze into beakers and then into a tube, 1 cm from the top of the tube.

This was followed by centrifugation at 1500-2000 rpm for two minutes. After removal from the centrifuge, the supernatant was poured into a funnel and the remaining sediment in the tube was made up to 1/3 of the tube with flotation Sheater´s solution. The contents were then mixed with the flotation solution, again into a total of 2/3 of the tube and centrifuged for two minutes at the same centrifugation values (see above), see Annex 4. Using a modified microbiological loop, a sample was taken from the surface of the tube contents with a loop and were painted on the degreased slides and then covered with a coverslip. Subsequently, a microscopic examination was performed using an optical light microscope, with an eyepiece magnification of 12.5x and an objective of 10x. Detailed morphological structure of coccidia and nematode oocysts were studied at 100x magnification.

3.5. Semiquantitative evaluation of the number of parasitic objects

The evaluation of the number of developmental stages of parasites was performed on the basis of the morphological structure of the present oocysts of coccidian and nematode eggs in the field of view of the light microscope according to the ovoscopic key for the determination of individual species of coccidia of the genus Eimeria in sheep and goats. The numbers of individual types of parasitic stages were always evaluated in 10

fields of view of the microscope (magnification of the eyepiece 12.5x and the objective 10x), where the value + one represented the number of oocysts from 1 to 4 parasitic objects; similar ++ two (5-10 parasitic objects) and +++ three (11 or more parasitic objects). Similarly, according to the helminthological key for sheep and goats, the presence of different developmental stages of helminths was detected.

3.6. Statistical analyzes

The obtained data were subjected to statistical analysis using the statistical program Statistica komplet CZ, version 12 (StatSoft, USA) and Microsoft Excel 10. The obtained and calculated data were processed using basic descriptive statistics of the sample, (arithmetic mean, standard deviation). The cause of variability in the data was determined using multifactor analysis (ANOVA). Then, statistically significant differences were analyzed by POST-HOC test, and the level of evidence alpha = 0.05 was chosen. Specifically, the Tukey's test was used.

4. Results

During the research, a total of 202 animal faecal samples were examined. GIN nematode eggs, namely *Trichuris* spp., *Trichostrongylus* spp., were detected in the examined samples and *Strongyloides* spp. and further developmental stages of oocysts *Eimeria* spp., as well. Due to the fact that the eggs of nematodes of individual representatives of nematodes were morphologically very similar in the diagnosis even within one species, therefore the determination in the coprological examination on the basis of morphology was difficult. In the selected Caribbean region, three species of Trichostrogylus spp. were represented, which differed slightly in size. *Trichostrongylus axei* (± 85µ), *Trichostrongylus colubriformis* (85-90µ), *Trichostrongylus vitrines* (> 90µ), as well. The represented species were diagnosed as GIN nematodes.

4.1. Evaluation of coprological examinations in sheep and goat farms

Occurrence of parasitic agents in breeding B

Sampling in breeding farm B, on the Sero Alejandro farm, took place in November 2020, when 24 ovine and caprine faecal samples were taken before and after the administration of the medicinal products (Vecoxan®). The results showed that positive samples were found in 100% in sheep and goats, similarly to the prevalence of helminthoses.

At the second sampling, after the application of the preparations (Vecoxan®), a lower incidence of developmental stages of nematodes was demonstrated in goats, when 67% of the collected faeces samples were positive. There was no observed parasites in animal control samples. The following developmental stages of species parasites were diagnosed: *Eimeria* spp., *Trichostrogylus* spp., *Strongyloides* spp., *Trichuris* spp..

Total number of samples (n)	Animal	Sampling	Overall prevalence of parasitosis (%)	Number of positive samples (n)	Occurence of coccidia (%)	Occurence of nematodes (%)
6	goat	Before	100%	6	100%	100%
6	goat	After	100%	6	100%	67%
6	sheep	Before	100%	6	100%	100%
6	sheep	After	100%	6	100%	100%
6	control	Before/After	0	0	0	0

Prevalence parazitóz v chovu C

Sampling in farm C, on the Sero Grandy farm, took place in November 2020, when 20 samples of sheep and goat faeces were taken before and after the administration of medicinal products (Vecoxan®). From the results in breeding C, 100% prevalence of parasitosis was demonstrated in all examined animals and the following developmental stages of parasites of the species were diagnosed: *Eimeria* spp., *Trichostrogylus* spp., *Strongyloides* spp., *Trichuris* spp. For control samples, there was no infection during testing of other animals.

Total number of samples (n)	Animal	Sampling	Overall prevalence of parasitosis (%)	Number of positive samples (n)	Occurence of coccidia (%)	Occurence of nematodes (%)
5	goat	Before	100%	5	100%	100%
5	goat	After	100%	5	100%	100%
5	sheep	Before	100%	5	100%	100%
5	sheep	After	100%	5	100%	100%
5	control	Before/After	0	0	0	0

Occurrence of parasitic agents in breeding D

In breeding D the results of a coprological examination of sheep and goats were evaluated on the Jaburibari farm. The number of samples taken was 18 and the sampling took place in November 2020. The first sampling, before the administration of the preparations, showed that all samples taken were parasitologically positive for *Eimeria* spp. and the prevalence of coccidiosis was 100% in both species of animals, in nematodes GIN it was 50% in sheep and goats negative. The second sampling was performed after the application of medicinal products (Emanox®), when there was a 20% increase in the prevalence of GIN parasitosis in goats and a 25% decrease in sheep. There was also a reduction in the prevalence of coccidiosis in sheep to 75% and in goats remained the same. There was no parasites present in the animal control samples. Representatives of the genera Eimeria, Trichuris and other genera GIN were mainly demonstrated in these samples.

Total number of samples (n)	Animal	Sampling	Overall prevalence of parasitosis (%)	Number of positive samples (n)	Occurence of coccidia (%)	Occurence of nematodes (%)
5	goat	Before	100%	5	100%	0%
5	goat	After	100%	5	100%	20%
4	sheep	Before	100%	4	100%	50%
4	sheep	After	75%	4	75%	25%
4	control	Before/After	0	0	0	0

Occurrence of parasitic agents in breeding E

Sampling took place at farm E, on the San Nicolas farm, in November 2020. 16 ovine and caprine manure samples were taken before and after the administration of the medicinal products (Emanox®). From the results in breeding E, 100% occurrence was demonstrated in all examined animals and species of parasites. Only in the second collection of faeces after application of the products was a decrease in the incidence of nematode GIN in sheep to 75% demonstrated. In control samples, there were no present developmental sages of parasites during testing of other animals. The following

developmental stages of species parasites were diagnosed: *Eimeria* spp., *Trichostrogylus* spp., *Strongyloides* spp., *Trichuris* spp..

Total number of samples (n)	Animal	Sampling	Overall prevalence of parasitosis (%)	Number of positive samples (n)	Occurence of coccidia (%)	Occurence of nematodes (%)
4	goat	Before	100%	4	100%	100%
4	goat	After	100%	4	100%	100%
4	sheep	Before	100%	4	100%	100%
4	sheep	After	100%	4	100%	75%
4	control	Before/After	0	0	0	0

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4.2. Intensity of parasitic GIN infection in goats and sheep before and after application of anticoccidials

Graph 1 showed the intensity of infection with the developmental stages of oocysts of *Eimeria* spp. before and after application of anticoccidials in small ruminants.

The left part of the graph shows the intensity of infection in goats before and after administration of plant extract E and veterinary medicinal product D. Before administration of the dose of extract E, the average value of infection intensity was 3 ± 0.00, after administration the value decreased to 1.8 ± 0, 84. Before administration of anticoccidial D, the intensity of infection was 2.82 ± 0.40, after administration, it decreased to 1.89 ± 0.78.

Statistical evaluation showed a significant reduction in the intensity of infection in goats, not only in plant extract E ($p = 0.014095$), but also in veterinary medicinal product D ($p = 0.002633$).

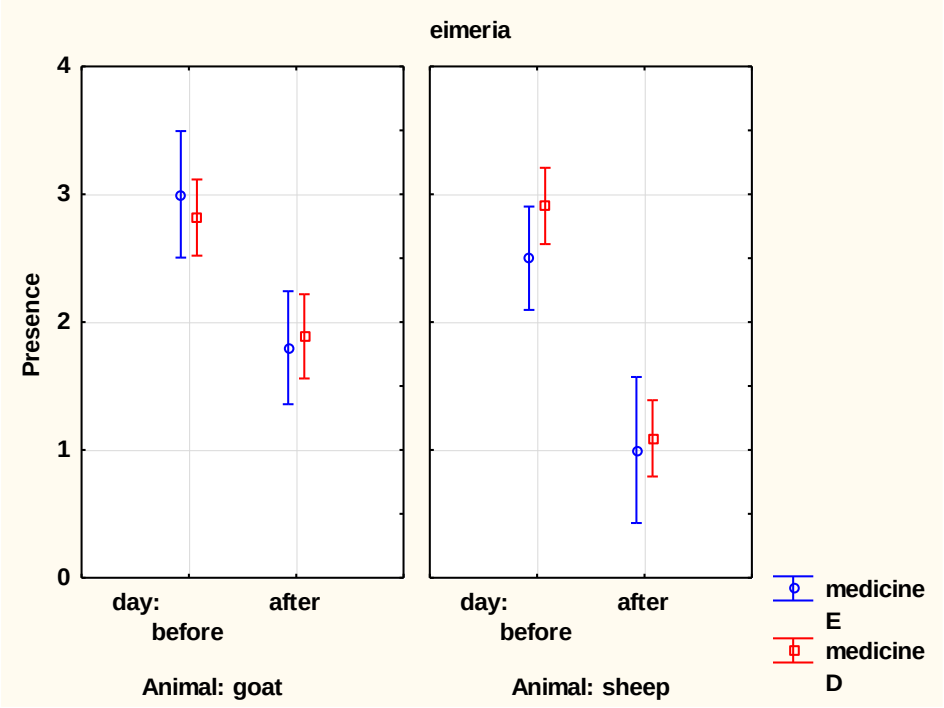
In the right part of the graph, the intensity of infection in sheep was monitored. The graph showed that the sheep had an average infection intensity of 2.50 ± 0.55 before the administration of plant extract E and a value of 1.0 ± 0.00 was found after administration. Prior to administration of veterinary medicinal product D, the intensity of infection in sheep was 2.82 ± 0.40, but decreased to 1.09 ± 0.30 after administration of this product.

There was also a statistically significant decrease in the intensity of infection in sheep, namely in both anticoccidials, when $p = 0.001896$ values were demonstrated for the plant extract and $p = 0.000135$ for the veterinary medicinal product.

Prior to the administration of both products, no statistically significant difference was found between goats and sheep in the intensity of infection with coccidia of *Eimeria* spp. There was no statistically significant difference in the intensity of infection between goats and sheep after administration of E. However, in the case of preparation D, a statistically significant difference ($p = 0.15239$) was found in the reduction in the intensity of infection between goats and sheep. There was a more significant decrease in the intensity of infection in sheep compared to goats.

It can be stated that both monitored anticoccidials E and D were effective and contributed to the reduction of the intensity of infection by the developmental stages of oocysts of the genus Eimeria. It has been statistically shown that in the treatment of ovine coccidiosis, a higher efficacy of the anticoccidial D has been found.

Graph 1: **Overall presence of developmental stages of coccidia - oocysts of *Eimeria* spp. in goats and sheep after application of anticoccidials (Emanox and Vecoxan)**



4.3. Average Weight

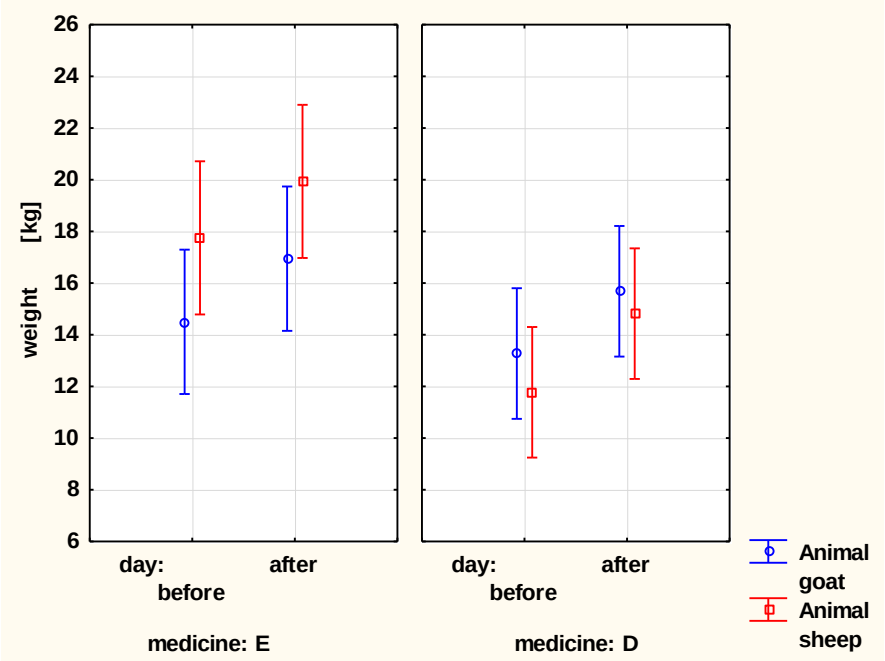
Graph No. 2: Average values of live weight of goats and sheep depending on the applied vet preparation. Graph 2 shows the average weights of sheep and goats before and after the application of medicine E and medicine D.

The left side of the graph shows the results after application of medicine E in goats and sheep. The average weight of goats before the application of composition E was 14.50 ± 4.53 kg, after the application of this preparation it was 16.94 ± 4.43 kg. There was no statistically significant difference in the average weight before and after administration of preparation E. In sheep, the average weight before application of composition E was found to be 17.75 ± 6.90 kg, after administration it was 19.94 ± 6.86 kg. There was no statistically significant difference in mean weight before and after administration of E in sheep. There was also no statistically significant difference between sheep and goats in either the initial average weight or the average weight after administration of E.

The right side of the graph shows the weight results after medicine D application in goats and sheep. The average weight of goats before application of composition E was 13.27 ± 2.90 kg, after application it was 15.68 ± 2.68 kg. There was no statistically significant difference in mean weight before and after administration of Formulation D. In sheep, the average weight before administration of Formulation D was 11.77 ± 2.46 kg, after administration of Formulation D it was 14.82 ± 1.87 kg. There was also no statistically significant difference in the average weight before and after administration of D in sheep. There was also no statistically significant difference between sheep and goats in either the initial average weight or the average weight after D administration.

It can be stated that for both products no significant differences in weight were found in sheep and goats before and after application.

Graph 2: Average values of live weight of goats and sheep depending on the applied preparation



4.4. Weight difference

Graph No. 3: The difference between the weight of goats and sheep depending on the application of medicine E and D.

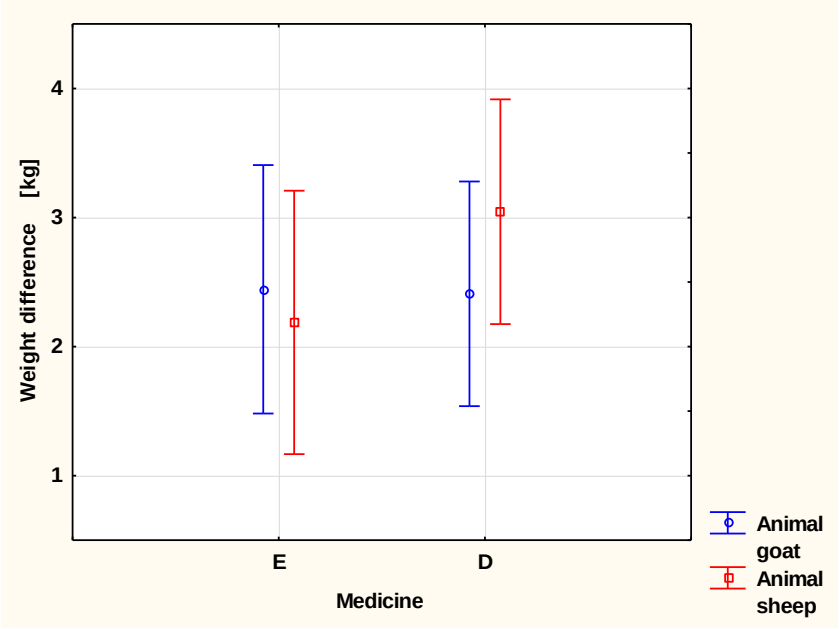
Graph 3 showed the differences in the weight of sheep and goats depending on the product used.

The difference in weight in goats before and after administration of preparation E was 2.44 ± 0.63 kg, in preparation D the difference in weight was 2.41 ± 1.59 kg. The differences were not significant for any of the products used.

The difference in weight in sheep before and after administration of preparation E was 2.19 ± 0.26 kg, in the case of preparation D a difference in weight of 3.05 ± 2.04 kg was found. Even in sheep, these ranks were not significant for any of the preparations used, although the highest difference between the initial and final weight was found in sheep after application of preparation D.

Also in the comparison of goats and sheep, no statistically significant difference was found in the differences in weight depending on the product used between the observed animal species, although in the case of use.

Graph No. 4: The difference between the weight of goats and sheep depending on the application of medicine E and D



5. Discussion

The research in this diploma thesis was part of a project focused on monitoring parasitosis in sheep and goats and subsequent testing of the effectiveness of an antiparasitic, with the active substance diclazuril and natural emanox extract. Riem van den Berg DVM, microbiologist, interim head of the veterinary service of Aruba. During the study, a total of 202 animal faeces samples were taken and coprologically examined, and the eggs of the trichostrongylid nematodes, *Trichuris* spp., *Strongyloides* spp. and *Eimeria* spp.

Due to the tropical conditions in Aruba, the sheep bred in selected farms were crossed by two breeds. The first breed Barbados blackbelly sheep was characterized by high fertility, in contrast to the second breed Dorper sheep, which was very resistant to heat, and so the island was created by crossing an ideal combination for breeding (Sambraus 2006). Boer goat breeding did not require crossbreeding, the breed was very efficient, with a slow metabolism, low water consumption and low food resources (Campbell and Donlan 2005).

The first major difference between animals can be observed in the localities that the animals preferred. Lowland suited sheep, unlike goats, which preferred higher terrain (Buurt 2012). In Aruba, the animals were kept together, but due to the fact that farms B, D, E were located in a large rural area, the animals had lowlands and the opportunity to move in higher terrain conditions. Farm C Sero Grandy was the only one located in an urban area, ie without the possibility of access to higher terrain. As these species of small ruminants had very similar requirements in terms of ethology and housing, nutrition and breeding, joint breeding of sheep and goats was easily implemented. At Farms B, D, the animals were kept together, when they were housed in pens at night and stayed on mixed pasture during the day. Unlike farms C and E, where the animals were kept together on a pasture with a shelter designed to protect them from adverse conditions. In my opinion, the great advantage was the joint breeding of animals and there could be a better use of pasture animals and reduce the need for fencing the pasture area.

Other differences between sheep and goats were reflected in their feeding behavior. Sheep grazed the ground layers of the stand permanently, while goats also grazed and grazed the middle and terminal parts of plants, shrubs and trees (Smith et

Sherman 1994). These differences could explain the remarkable ability of goats to detoxify exogenous chemicals, including anthelmintics, and their reduced ability to develop an effective response against gastrointestinal parasites (Hoste et al. 2011). By favoring the use of nutrients by the immune system, sheep could be deprived of the nutrients needed to maintain resistance to parasites. Another difference was the excretion and accumulation of parasitic eggs from the animal´s body. Adult sheep excreted eggs extensively from the body, but in goats they tended to accumulate within the gastrointestinal tract and eggs were excreted continuously, to a lesser extent (Huntley et al. 1995). In addition, in breeding goats, the level of infection was almost the same in adults and young animals, in contrast to sheep, where the manifestations of infection in adult females were much less severe (Hoste et al. 2008). For safe breeding of young individuals, especially lambs, which were very susceptible to infections at this age, it was recommended to divide them according to age categories. In Aruba, in selected localities, the animals were not divided according to age categories, not even on a single monitored farm. This standard practice used to be much better established in European farms than in this tropical Caribbean locality, for both economic and spatial reasons.

Climatic conditions in the area determined the species presence of parasites and their life cycle. Temperature affected the rate of development and survival of wild developmental stages of parasites. The moisture prevented the larvae from drying out and allowed the larvae to migrate through the vegetation. Vegetation density and height have influenced the potential for the spread of pathogenic parasites (Torres-Acosta et Hoste 2008). The infectious burden of GIN on pastures has been reduced by following biosecurity rules, ie by controlling the sources of infection leading to recovery (Houdijk et al. 2012). However, the third developmental larval stage of nematodes was prone to prolonged drought, so humid weather in autumn and winter was associated with an increased annual incidence of GIN in goats and sheep.

The use of rotary grazing helped to heal pastures from the developmental stages of coccidia and GIN. The longer the pasture remained fallow, the less it could be contaminated by larval stages. A rotary grazing system should be introduced prior to antiparasitic therapy to control external biosecurity (Stromberg et Averbeck 1999). According to Host et Torres-Accosta (2011), farmers should not prioritize the control of helminthiasis as a top priority for rotary grazing, which could have a negative impact on

animal productivity and health. Given the persisting differences in the survival of L_3 dominant species in temperate and tropical climates, grazing rotation would be the starting point for GIN control only in hot and humid climates, making it an ideal choice for this tropical site where no grazing control procedures have been established. The only way to eliminate the developmental stages of parasites on pastures was regular removal of excrement. I would therefore suggest that, in these cases, rotary grazing systems be put in place so that forage on the pasture is used efficiently by the animals (Hoste et Torres-Accosta 2011). Another possible successful means of ensuring pasture biosecurity could be to implement an alternative grazing strategy. Barger (1999) stated that two or more host species in a given environment did not share common parasite species and that interspecies rotation could be a successful means of increasing antiparasitic control. Grazing small ruminants with cattle, or small ruminants with horses, or breeding horses with cattle could be a good choice. In sheep and goat rotation, this method would not be successful due to the fact that both species were always infected with the same species of parasites.

Meeusen et al. (2005) emphasized the economic severity of grazing parasites, especially nematodes, which increased with the onset of anthelmintic resistance to these parasites. According to the above authors, the detection of specific antibodies against grazing parasitosis was burdened by the fact that gastrointestinal parasites within the host body moved very quickly from one larval stage to another. Due to these facts, no general measures and application of anthelmintics have been carried out in Aruba so far. Only on farm A was it administered to sick animals. Therefore, this situation did not occur on the island of Aruba, and there was no resistance to any anthelmintics on selected farms. The main mechanism by which animals acquired immunity against parasites was so-called reinfection, and the effector mechanisms of the ruminant immune system were thus able to adapt to the different life cycles of individual parasites (Meeusen et al. 2005).

The management of sheep and goat breeding on selected farms was positively evaluated in terms of ensuring the natural needs and welfare conditions of animals. The demands and requirements for the breeding of small ruminants resulted from the valid legislation on livestock breeding, as well as from the principles of organic farming and ethological studies, which were met on farms.

Sheep and goats were able to exercise their natural behavior thanks to a system of free grazing, where they had enough space for movement and grazing behavior and received a varied ration. The hay area, feed troughs and feed supplements have been designed and located to prevent injury or damage to the eyes and the sheep and goats are not endangered by falling feed bales. Drinking bowls were designed and placed to minimize the possibility of fecal or urine contamination or to prevent water spillage to prevent injury. At least once a day, they were inspected and kept clean by the breeder, and more often during extreme weather fluctuations.

Sheep and goats could only exceptionally be bred individually. Therefore, they were housed in group pens, only during the birth and lactation of young were housed on farms B and D in individual pens. The pastures on the pastures took place in sheep and goats, adapted to the given environment and local conditions, ie mainly on farms C and E. The entrances and exits of the buildings and enclosures were kept in good condition, without obstacles and were adjusted to avoid injury to the animals. The enclosure has been properly designed and maintained to prevent escape of animals and the risk of injury to sheep or goats. When using wire mesh, the fence was often inspected and kept taut so that it would not get caught, especially horned sheep or horned goats.

Special attention was paid to the treatment of hooves and preventive measures were taken at regular intervals to reduce the spread of hoof rot and other infections. The care also focused on the maintenance of tools in working order, which were used for cutting and marking animals and also for the application of antiparasitic preparations. The number of medicinal products also corresponded to the number of animals treated by weight and their age categories.

In farms C and E, where the animals were kept in extensive conditions, the animals and breeding facilities were inspected at least once a week. However, inspections were carried out more frequently when the welfare of sheep or goats was endangered, especially at the time of parturition or at a time of increased risk of being attacked by predators; wild dogs were present in these areas. In the case of sheep and goats, the need for water in pastures was met on a daily basis, either by providing them with water of sufficient quantity and quality, or only in feed, with a sufficient water content, or a combination of both.

The occurrence and process of infection by the developmental stages of parasites was influenced by several factors, depending on the age of the animals, the type of breeding or the climatic conditions. In the past, excessive use of anthelmintics has been a common solution for treating infected animals, leading to an increase in resistant nematode strains, which have become a worldwide problem today. The current trend has become targeted treatment or targeted selective treatment of clinically ill animals (Novák et al. 2012; Novák et al. 2017). In my opinion, it was important to detect possible parasitic infections on selected farms as soon as possible and increase biosecurity. One of the ways to increase biosecurity was effective control by the management, when sampling and application of anthelmintics were carried out at the appropriate time, in cooperation with veterinarians.

Basic biosecurity measures, on the other hand, have been better applied when animals have been housed indoors and kept on a rotating basis (Stromberg and Averbeck 1999). Adherence to these measures was complicated in animals on farms C and E, where they were housed only on sheltered pastures. Biosafety has been affected by a number of factors. In the case of mutual contact of a larger number of animals, the inclusion of new animals in the herd has become a significant risk (Novák et al. 2017). Therefore, preventive measures included thorough health inspections of the animals before purchase and transport and obtaining essential information on the origin of the animals, the health status of the herd and the epidemiological situation in the region where the purchased animals came from. Furthermore, it would be necessary to ensure the housing of new animals outside the existing herd, in the so-called quarantine (min. 14 days). The human factor played an important role, as the care staff should follow basic hygienic procedures during contact with animals. Similarly, O'Mahony (2015) described that the entry of domestic or wild animals (dogs, cats, game, etc.) should be prevented. confinement of animals in individual pens overnight.

It was found to be 100% during the study occurrence of coccidia on all sheep and goat farms. Ruitenbeek (2016) in his study carried out on the island of St. Eustatius in the Caribbean, described the occurrence of *Eimeria* spp. in faeces in 49.1% of sheep and 34.5% of goats, while Borgsteede (1996) in his study in the Netherlands reported 82% of sheep and 78% of goats in *Eimeria* spp. Author Kahan (2013) showed a 69% prevalence of coccidiosis in sheep in the United States. The results of research from Mexico (Alcala-

Canto 2020) showed the occurrence of sheep coccidia, especially *E. faurei* in 58.64% and goats in 49.90%. The research of Macedo et. al (2019) also confirmed the findings of coccidia of different species of *Eimeria* spp. Sheep were positive in 74% and goats in 66%. Thus, it can be stated that in this pilot study a higher prevalence of coccidiosis was recorded, which was close to the study from the Netherlands (Borgsteede 1996). The results of the Taylor and Catchpole (1994) study showed that pups aged three weeks to six months were most susceptible. The prevalence of ovine and caprine coccidiosis was relatively high and could be as high as 100% in juveniles, which explained the achieved coccidial capture in selected farms from various localities of Aruba, from where the youngest individuals were selected from farms for therapeutic purposes.

Part of this work was also monitoring the occurrence of developmental stages, ie nematode eggs, which reached values of 100% on farms B, C and E, while on farm D no infection was detected in goats and only a small capture of helminth eggs in sheep (50%). From a qualitative point of view, the occurrence of at least one species of monitored GIN helminths was found in most samples, so-called polyinfections were mostly detected and several species of nematodes were detected, eg *Strongylus* spp., *Trichuris* spp., *Trichostrongylus* spp. Scientific research Ruitenbeek (2016), which was carried out on the nearby island of St. Eustatius demonstrated the prevalence of coccidiosis in sheep and goats, with species representatives of *Strongylus* spp. occurring in 82.7% of sheep and even in 91.0% of goats. Another important species was *Strongyloides papillosus* (19.2% in sheep, 22.1% in goats), as well as tapeworm eggs (7.6% in sheep, 12.1% in goats) and, to a lesser extent, *Trichuris ovis* (5.1% in sheep, 9.7% in goats). In this study from Mexico, Olivas-Salazar et. al. (2018) reported a total incidence of trichostrongylid nematodes of 85.5% in sheep and 88.9% in goats. In Brazil, the Lins study (2019) showed an 84.7% incidence in sheep. The most common species have become nematodes *Trichostrongylus* spp. (13.8%), *Oesophagostomum* spp. (3.6%), *Strongyloides* spp. (2.4%). In this study, higher nematode growths were also recorded on the island of Aruba, compared to the above countries, although it was also significant on the island of Eustatius in the Caribbean. In a study by Host et al. (2008) revealed a higher level of parasitic infection in goats than in sheep in common grazing. These results supported the hypothesis of a lower immune response to grazing goats and susceptibility to pathogenic helminths, compared to sheep.

In sheep and goats, a number of species of coccidia have been parasitic, such as *Eimeria arloingi, Eimeria ovinoidalis, E. crandallis, E. granulosa, E. faurei* (See Annex 1). Coccidiosis occurred when the infectious feed and water were ingested and the animals were licked. The ingested oocysts penetrated into the cells of the intestinal mucosal epithelium (Haták et al. 2008). Most species of coccidia of the genus Eimeria caused subclinical coccidiosis, ie diarrheal disease, especially in young individuals. Highly pathogenic species included *Eimeria arloingi* and *Eimeria ovinoidalis*, which caused serious clinical diseases with symptoms of so-called hemorrhagic diarrhea. There was also the formation of intestinal lesions, cachexia and its exhaustion, often with fatal consequences, especially in young. The acute form was manifested by high fevers (+ 40 ° C), diarrhea with mucus and blood (Chroust et al. 1998; Najdrowski 2005, Kváč et al. 2006, Vadlejch 2013). No such acute symptoms appeared in the herd studied. Mild diarrhea did not correspond to an increased incidence of oocysts, but appeared after weaning and transition of animals to pasture, ie after a fundamental change in the feed ration, when milk and hay were replaced by fresh grass.

The gastrointestinal nematode *Strongyloides papillosus* was a parthenogenic female present in the small intestine of sheep. The infection started with the intake of infectious larvae (grade L_3) orally, with food, water (passive) or percutaneous (active) infection with L_3 larvae (Šibalić and Cvetković 1996). In young individuals, *Strongyloides* spp. sudden death syndrome due to heart failure (Abott and Lewis 2005). Trichostrongylus (*Trichostrongylus colubriformis*, *Trichostrongylus vitrinus*, *Trichostrongylus rugatus*) usually lived in the digestive tract, especially in the small intestine, of domesticated and wild herbivorous animals with their heads inserted into the mucosa. The infection occurred by accidental ingestion of vegetation-contaminated third-degree infectious larvae. The main symptoms were anorexia, persistent diarrhea, weight loss, and venous atrophy (or stunting of the villi), which led to impaired digestion and malabsorption, with protein loss occurring on the damaged mucosa (Mark 2014). Of the genus Trichuris, *Trichuris globulosa*, *Trichuris discolor* and *Trichuris ovis* were the most parasitic in sheep and goats. Adults induced juvenile bleeding by permanent movement in the intestinal mucosa. Only the young population showed clinical signs, the elderly did not manifest the symptoms. Dehydration, weight loss, anemia and diarrhea took place, when adult worms could also appear (Hofírek et al. 2009). In the herd we studied, gastrointestinal nematodes showed clinical signs, especially in the young population,

when diarrhea and weight loss occurred. There were no sudden deaths due to infection with these nematode species. Nematodes of the species *Strongyloides papillosus* have not been coprologically proven.

In the pilot year, the research was initially focused on parasitological monitoring and the main goal was to determine the occurrence of parasite species in the faeces of sheep and goats using coprological methods and to select suitable sites for the next phase of the project. Monitoring was performed by collecting mixed samples at all selected localities. Furthermore, the occurrence of parasitic species was recorded and according to the appropriate breeding management, suitable farms were selected for the next phase. In the following year, the final output of the research was the determination of antiparasitic efficacy in selected localities. Two antiparasitic veterinary medicinal products were selected for the research, one of chemical origin (Vecoxan®) and the other of alternative, biological origin (Emanox®). In the final research, individual sampling was performed on the basis of monitoring and then from the youngest individuals on the farm, who belonged to the risk group.

Vecoxan® oral suspension, with the active substance diclazuril 2.5 mg/ml, was classified as an anticoccidial of benzeneacetonitrile groups, y without antimicrobial activity, with anticoccidial activity against *Eimeria* spp. The active ingredient diclazuril was chosen for its non-invasive damage to intestinal microbiots (formerly called microflora), in contrast to sulfonamide, which could damage them after application. Additional supportive treatment would be necessary to improve the course of the disease, in the case of confirmed clinical coccidiosis in individual animals already showing symptoms of diarrhea, as diclazuril had no antimicrobial effects. However, frequent and repeated use of these antiparasitic drugs could lead to the onset of resistance in the parasites concerned. Therefore, another alternative preparation Emanox® was chosen, which was due to its varied composition (mixture of plant extracts) more resistant to the development of resistance than conventional chemical coccidiostats. Emanox® was considered a natural product and therefore it could be used without restriction on organic farms to produce organic / eco products. It did not have a withdrawal period and could therefore be used regardless of the time of slaughter. Depending on the dose, it was used to both prevent and treat coccidiosis. Emanox® was applied to water for 30 days, in contrast to diclazuril, which was administered once and orally to all selected animals.

Graph 1 shows the intensity of parasitic infection of developmental stages of coccidia in goats and sheep before and after application of anticoccidials. There was no statistically significant difference in the intensity of infection between goats and sheep after administration of Emanox®. However, for diclazuril, there was a statistically significant difference in the reduction in infection intensity between goats and sheep. There was a more significant decrease in the intensity of infection in sheep compared to goats. It could be stated that both monitored anticoccidials Emanox® and diclazuril were effective and contributed to the reduction of the intensity of infection by the developmental stages of oocysts of the genus Eimeria. However, it has been statistically proven that in the treatment of coccidiosis in sheep, a higher efficacy of the anticoccidial diclazuril was found, so I would recommend the anticoccidial diclazuril for selected farms, which proved to be more effective. As they have not used any treatment measures in this locality so far, there is no need to worry about possible resistance to this anticoccidial. Sampling was performed on all farms in control groups of sheep and goats, for a comprehensive determination of the effectiveness of the products in the monitored groups.

Graph 2 shows the average weights of sheep and goats before and after the application of Emanox® (medicine name Emanox®) and diclazuril (medicine Vecoxan®). No significant differences in weight were found between sheep and goats in both products before and after application. Graph 3 showed the differences in the weight of sheep and goats depending on the product used. There was also no statistically significant difference in the differences in weight depending on the product used between the observed animal species. It could be said that the application of both products did not have a major effect on their weight. To increase the weight of the animals, the products would have to be used regularly, which could lead to a reduction in the incidence of developmental stages of parasites, possibly even their elimination.

6. Conclusions

During the research, monitoring was carried out and later individual sampling of sheep and goat droppings on the monitored farms on the island of Aruba. The flotation method according to Sheather was used in the laboratory, which made it possible to detect even a small number of parasitic oocysts and eggs. The occurrence of species representatives of cocidia and GIN nematodes in different types of farms on this Caribbean island was monitored and then a comparison of the achieved results with the adjacent states was performed. In animals infected with coccidial oocysts, classical veterinary medicinal products were administered at the same time and plant extracts with declared efficacy against coccidia were also used for therapy. The most common findings in sheep and goats were various parasitic species of *Eimeria* spp. and eggs of gastrointestinal nematodes. The intensity of the infection decreased after application and breeders were recommended to introduce regular control and treatment measures. To reduce the prevalence of parasitosis, it is necessary to introduce preventive procedures, observe zoohygienic and biosecurity in different types of animal husbandry. Monitoring of parasitosis, ie coccidiosis and helminthosis, regular sampling and subsequent coprological examinations, including the application of antiparasitics based on the results of the coprological examination, could thus guarantee the health of the animals. For farm breeding and coprological findings, breeders could administer antiparasitics in the form of a lick or add them to medicated feed. By keeping breeding and medical records on the application of antiparasitics in individual animal experiments (not only the implementation of widespread application of veterinary medicinal products in animal husbandry) it is possible to prevent the onset of resistance to the relevant antiparasitics. It was important to regularly monitor the health of the animals, ensure monitoring of other infectious pathogens and apply the principles of biosecurity in farms, which can be done in cooperation with breeders and the State Veterinary Service on the island of Aruba.

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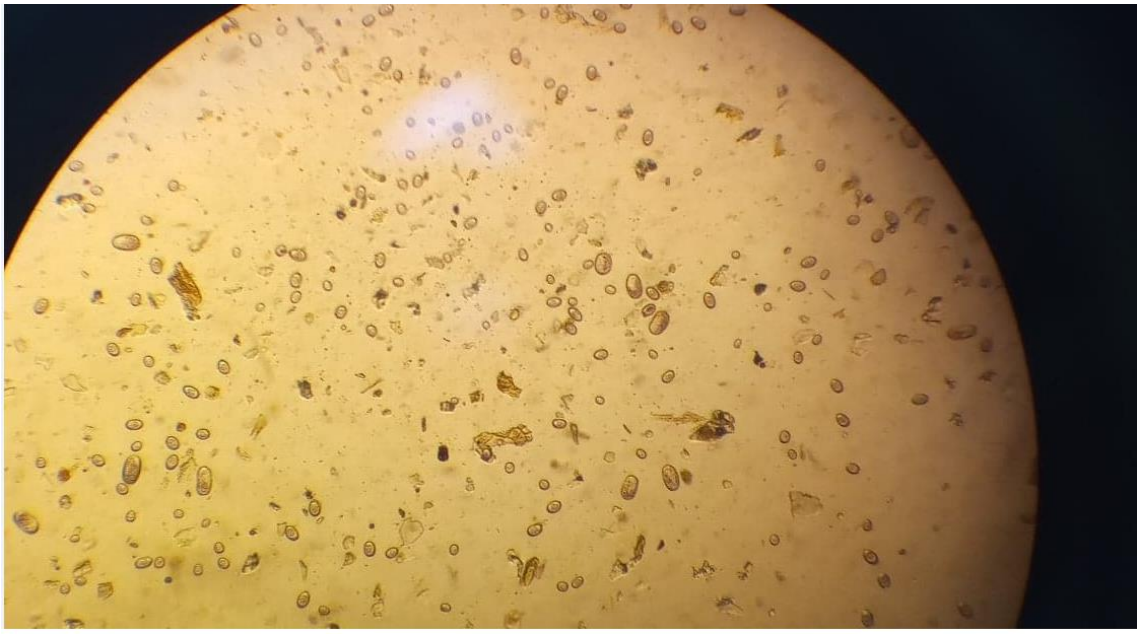
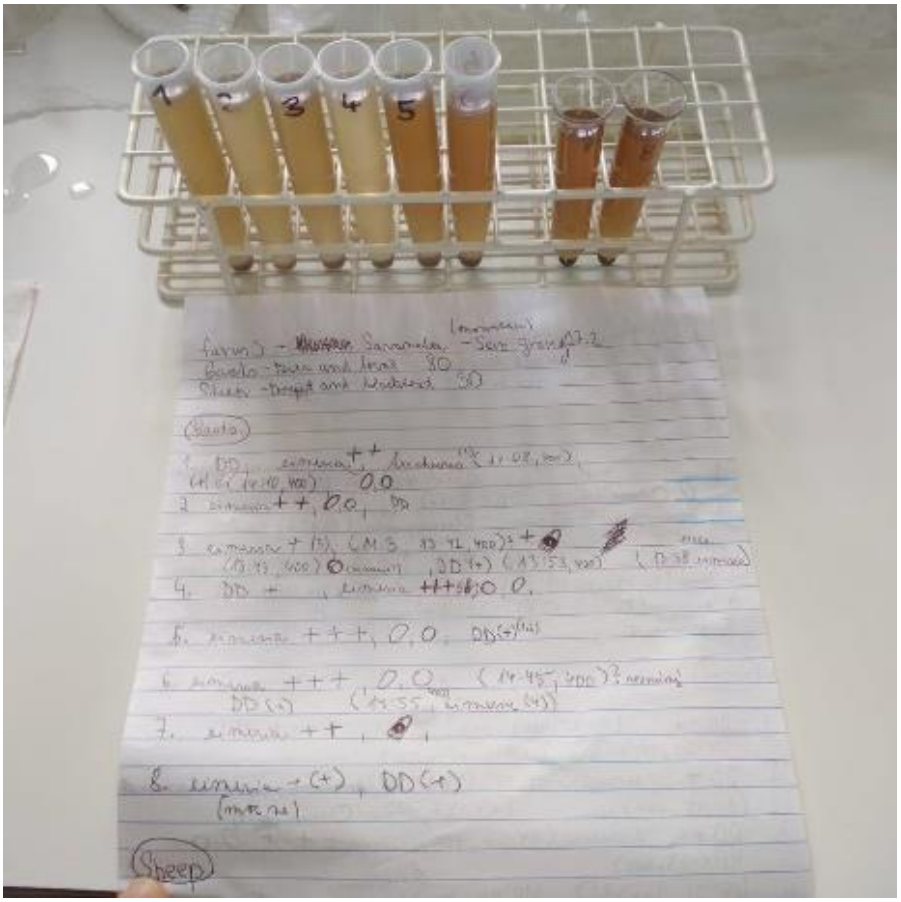
Appendices

List of the Appendices:

Appendix 1: Eggs of *Eimeria* spp. (Photo Dominika Roudná)

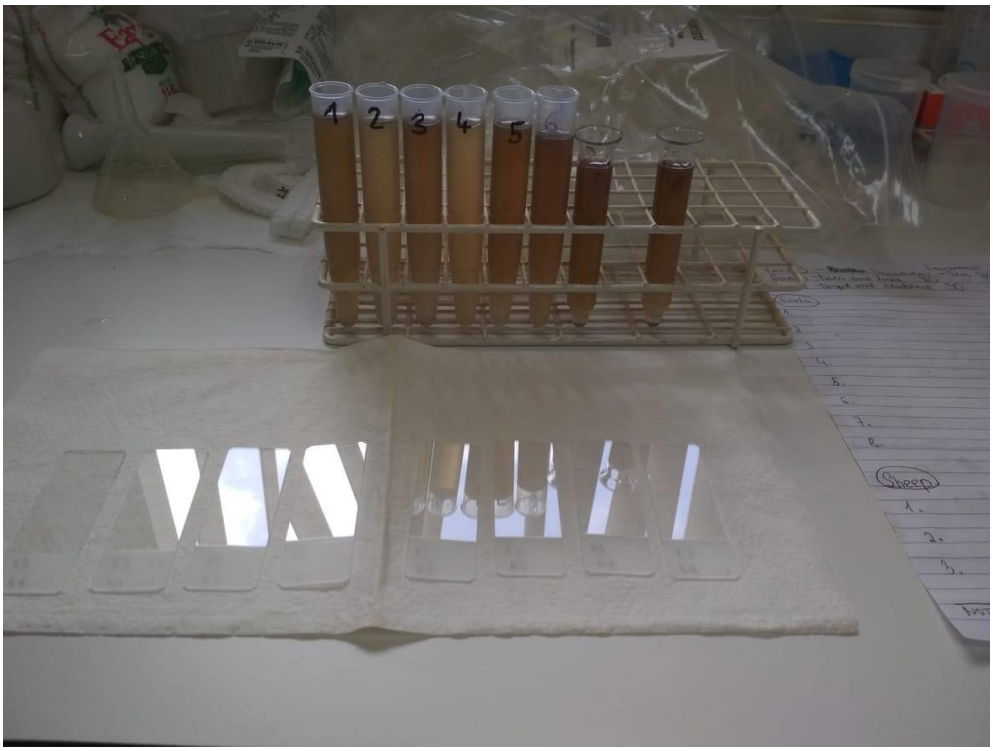


Appendix 2: Working protocol with prepared samples (Photo Dominika Roudná)



Appendix 3: Mortar with pestle (Photo Dominika Roudná)

Appendix 4: Prepared samples (Photo Dominika Roudná)



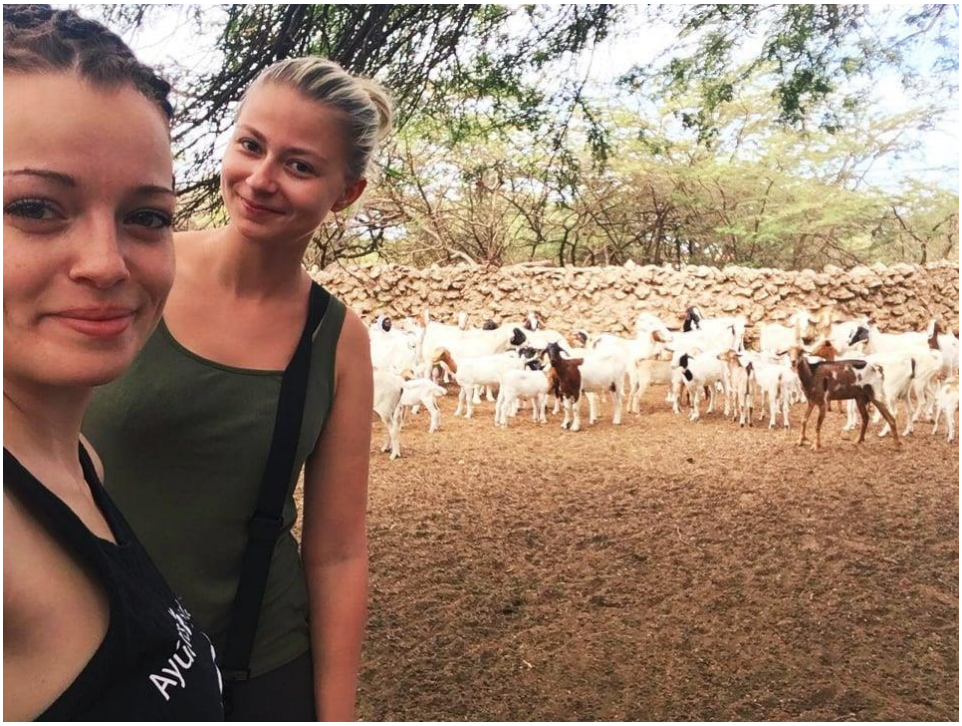
Appendix 5: Filtering the sample through gauze (Photo Dominika Roudná)

Appendix 6: Laboratory material – Flotation solution (Photo Dominika Roudná)

Appendix 7: Final microscopy (Photo Dominika Roudná)

Appendix 8: Centrifuge (Photo Dominika Roudná)

Appendix 9: Farm B - Sero Alejandro (Photo Dominika Roudná)



Appendix 10: Farm C - Sero Grandy (Photo Dominika Roudná)

Appendix 11: Farm D - Jaburibari (Photo Dominika Roudná)

Appendix 12: Farm E – San Nicolas (Photo Dominika Roudná)

Appendix 13: Collection of samples on the farm B – Sero Alejandro – 15.11-15.12 2020 (Photo Dominika Roudná)

Sero Alejandro	Animal	Identification n.	height	weight	age	Eimeria	GIN	Trichuris
1. Collection	goat	122121	65cm	17kg	1year	+++	++	
Before	goat	122122	57cm	15kg	1year	+++	+	+
	goat	122123	57cm	17kg	1year	++	++	
	goat	122124	49cm	14kg	1year	+++	+	
	goat	122125	40cm	7kg	4m	+++	+	
	goat	122126	58cm	13kg	1year	++	++	
	sheep	122127	52cm	11kg	8m	+++	+	
	sheep	122128	56cm	12kg	8m	++	+	
	sheep	122129	60cm	15kg	8m	+++	++	
	sheep	122130	58cm	14,5kg	8m	+++	+	
	sheep	122131	55cm	12kg	8m	+++	++	
	sheep	122132	57cm	16kg	8m	+++	+	
2. Collection	goat	122121	67cm	17,5kg	1year	+	+	
After	goat	122122	61cm	18kg	1year	+	+	
	goat	122123	59cm	18,5kg	1year	++	++	
	goat	122124	51cm	15,5kg	1year	+	+	
	goat	122125	44cm	9kg	4,5m	+		
	goat	122126	60cm	13,5kg	1year	+		
	sheep	122127	54cm	12kg	8,5m	+	+	
	sheep	122128	57cm	13kg	8,5m	+	+	
	sheep	122129	62cm	16,5kg	8,5m	+	+	
	sheep	122130	60cm	16kg	8,5m	+	+	
	sheep	122131	57cm	13,5kg	8,5m	+	+	
	sheep	122132	59cm	17,5kg	8,5m	++	+	

Appendix 14: Collection of samples on the farm C – Sero Grandy - 15.11-15.12 2020 (Photo Dominika Roudná)

Noord	Animal	Identification n.	height	weight	age	Eimeria	GIN	Trichuris
1. Collection	goat	124121	57cm	14kg	9m	+++	++	
Before	goat	124122	48cm	12kg	9m	+++	++	+
	goat	124123	55cm	13kg	9m	+++	+	
	goat	124124	51cm	14kg	9m	+++	+++	+
	goat	124125	52cm	10kg	8m	+++	++	+
	sheep	124126	50cm	10kg	9m	+++	+	
	sheep	124127	54cm	10kg	9m	+++	++	
	sheep	124128	60cm	11kg	9m	+++	+++	+
	sheep	124129	51cm	8kg	9m	+++	++	+
	sheep	124130	55cm	10kg	9m	+++	+	+
2. Collection	goat	124121	62cm	18kg	10m	++	++	
After	goat	124122	57cm	15kg	10m	++	++	+
	goat	124123	56cm	15,5kg	10m	+++	+	
	goat	124124	57cm	16kg	10m	++	+	
	goat	124125	65cm	16kg	9m	+++	+	+
	sheep	124126	58cm	15,5kg	10m	+	+	
	sheep	124127	61cm	16kg	10m	+	+	
	sheep	124128	67cm	15,5kg	10m	+	+	
	sheep	124129	64cm	12kg	10m	+	+	
	sheep	124130	61cm	15,5kg	10m	+	+	

Appendix 15: Collection of samples on the farm D – Jaburibari - 15.11-15.12 2020 (Photo Dominika Roudná)

Jaburibari	Animal	identification n.	height	weight	age	eimeria	GIT	trichuris
1. Collection	goat	121123	36cm	9,5kg	2m	+++		
	goat	121124	44cm	10kg	2m	+++		
	goat	121125	62cm	18,5kg	4m	+++		
	goat	121126	57cm	17kg	3m	+++		+
	goat	121127	56cm	16,5kg	3m	+++		
	sheep	121128	58cm	20kg	4m	++		
	sheep	121129	60cm	22kg	4m	++	+	
	sheep	121130	68cm	25kg	4m	++	+	+
	sheep	121131	67cm	28kg	4m	++		+
2. Collection	goat	121123	38cm	12kg	3m	+	+	
	goat	121124	45cm	11,5kg	3m	+		
	goat	121125	65cm	21kg	5m	+		
	goat	121126	61cm	20kg	4m	+		+
	goat	121127	60cm	19,5kg	4m	+		
	sheep	121128	61cm	22kg	5m	+		
	sheep	121129	63cm	24kg	5m	+		
	sheep	121130	70cm	27,5kg	5m		+	+
	sheep	121131	70cm	30kg	5m	+		+

Appendix 16: Collection of samples on the farm E – San Nicolas - 15.11-15.12 2020 (Photo Dominika Roudná)

San Nicolas	Animal	identification n.	height	weight	age	eimeria	GIT	trichuris
1. Collection	goat	123121	65cm	19kg	1year	+++	++	+
	goat	123122	52cm	11kg	3m	+++	+++	
	goat	123123	46cm	9kg	2m	+++	+	+
	goat	123124	67cm	20kg	1year	+++	++	+
	sheep	123125	45cm	10kg	2m	+++	+	
	sheep	123126	49cm	11kg	2m	++	+	+
	sheep	123127	50cm	12kg	2m	+++	++	+
	sheep	123128	56cm	14kg	3m	+++	++	+
2. Collection	goat	123121	67cm	21kg	1year	+	+	+
	goat	123122	56cm	14kg	4m	++	++	
	goat	123123	50cm	12kg	3m	+++	+	+
	goat	123124	69cm	21,5kg	1year	++	++	+
	sheep	123125	49cm	12,5kg	3m	+	+	
	sheep	123126	53cm	13kg	3m	+		+
	sheep	123127	55cm	14kg	3m	+	+	
	sheep	123128	58cm	16,5kg	4m	+	+	+

Appendix 17: Tukey´s HSD test - Results graph 1 (Photo Dominika Roudná)

Tukeyův HSD test; proměnná eimeria (Výsledky excel 1)
Přibližné pravděpodobnosti pro post hoc testy
Chyba: meziskup. PČ = ,24314, sv = 52,000

Č. buňky	medicine	day	Animal	{1} 3,0000	{2} 2,5000	{3} 1,8000	{4} 1,0000	{5} 2,8182	{6} 2,9091	{7} 1,8889	{8} 1,0909
1	E	before	goat		0,765000	0,014095	0,000185	0,998298	0,999984	0,009917	0,000135
2	E	before	sheep	0,765000		0,290378	0,001896	0,905220	0,727498	0,286818	0,000149
3	E	after	goat	0,014095	0,290378		0,356348	0,007879	0,002820	0,999981	0,156556
4	E	after	sheep	0,000185	0,001896	0,356348		0,000148	0,000139	0,144555	0,999992
5	D	before	goat	0,998298	0,905220	0,007879	0,000148		0,999863	0,002633	0,000135
6	D	before	sheep	0,999984	0,727498	0,002820	0,000139	0,999863		0,000781	0,000135
7	D	after	goat	0,009917	0,286818	0,999981	0,144555	0,002633	0,000781		0,015239
8	D	after	sheep	0,000135	0,000149	0,156556	0,999992	0,000135	0,000135	0,015239	

Popisné statistiky (Výsledky excel 1)

Efekt	Úroveň Faktor	Úroveň Faktor	Úroveň Faktor	N	eimeria Průměr	eimeria Sm.odch.	GIT Průměr	GIT Sm.odch.
Celkem				60	2,183333	0,873172	1,416667	0,590652
medicine*day*Animal	E	before	goat	4	3,000000	0,000000	2,000000	0,816497
medicine*day*Animal	E	before	sheep	6	2,500000	0,547723	1,333333	0,516398
medicine*day*Animal	E	after	goat	5	1,800000	0,836660	1,400000	0,547723
medicine*day*Animal	E	after	sheep	3	1,000000	0,000000	1,000000	0,000000
medicine*day*Animal	D	before	goat	11	2,818182	0,404520	1,727273	0,646670
medicine*day*Animal	D	before	sheep	11	2,909091	0,301511	1,545455	0,687552
medicine*day*Animal	D	after	goat	9	1,888889	0,781736	1,333333	0,500000
medicine*day*Animal	D	after	sheep	11	1,090909	0,301511	1,000000	0,000000

Appendix 18: Tukey´s HSD test - Results graph 2 (Photo Dominika Roudná

Tukeyův HSD test; proměnná weight (Výsledky excel 1)
Přibližné pravděpodobnosti pro post hoc testy
Chyba: meziskup. PČ = 17,665, sv = 70,000

Č. buňky	medicine	Animal	day	{1} 14,500	{2} 16,944	{3} 17,750	{4} 19,938	{5} 13,273	{6} 15,682	{7} 11,773	{8} 14,818
1	E	goat	before		0,918977	0,753823	0,151939	0,998001	0,998454	0,833637	1,000000
2	E	goat	after	0,918977		0,999929	0,822759	0,526593	0,997608	0,128719	0,949012
3	E	sheep	before	0,753823	0,999929		0,966355	0,312463	0,963074	0,059089	0,804276
4	E	sheep	after	0,151939	0,822759	0,966355		0,022700	0,376918	0,002106	0,165928
5	D	goat	before	0,998001	0,526593	0,312463	0,022700		0,878507	0,990330	0,988461
6	D	goat	after	0,998454	0,997608	0,963074	0,376918	0,878507		0,375656	0,999730
7	D	sheep	before	0,833637	0,128719	0,059089	0,002106	0,990330	0,375656		0,687672
8	D	sheep	after	1,000000	0,949012	0,804276	0,165928	0,988461	0,999730	0,687672	

Popisné statistiky (Výsledky excel 1)

Efekt	Úroveň Faktor	Úroveň Faktor	Úroveň Faktor	N	weight Průměr	weight Sm.odch.
Celkem				78	15,32692	4,661132
medicine*Animal*day	E	goat	before	9	14,50000	4,534589
medicine*Animal*day	E	goat	after	9	16,94444	4,426091
medicine*Animal*day	E	sheep	before	8	17,75000	6,902381
medicine*Animal*day	E	sheep	after	8	19,93750	6,863139
medicine*Animal*day	D	goat	before	11	13,27273	2,901410
medicine*Animal*day	D	goat	after	11	15,68182	2,685821
medicine*Animal*day	D	sheep	before	11	11,77273	2,463368
medicine*Animal*day	D	sheep	after	11	14,81818	1,874470

Appendix 19: Tukey´s HSD test - Results graph 3 (Photo Dominika Roudná)

	Tukeyův HSD test; proměnná weight difference (Výsledky excel 1) Přibližné pravděpodobnosti pro post hoc testy Chyba: meziskup. PČ = 2,0236, sv = 35,000					
Č. buňky	Animal	medicine	{1} 2,4444	{2} 2,4091	{3} 2,1875	{4} 3,0455
1	goat	E		0,99994	0,98224	0,78372
2	goat	D	0,99994		0,98686	0,72213
3	sheep	E	0,98224	0,98686		0,57042
4	sheep	D	0,78372	0,72213	0,57042	

	Popisné statistiky (Výsledky excel 1)				
Efekt	Úroveň Faktor	Úroveň Faktor	N	weight difference Průměr	weight difference Sm.odch.
Celkem			39	2,55128	1,40391
Animal*medicine	goat	E	9	2,44444	0,63464
Animal*medicine	goat	D	11	2,40909	1,59402
Animal*medicine	sheep	E	8	2,18750	0,25877
Animal*medicine	sheep	D	11	3,04545	2,04272

Appendix 20: Sporulated oocysts of the principal species of *Eimeria* in sheep and goats (Eckert et. al 1995)